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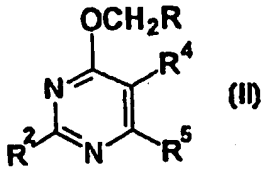

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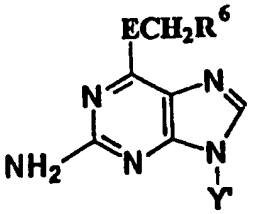

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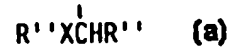

(a)

(57) Abstract

The invention provides compounds exhibiting the ability to deplete O6-alkylguanine-DNA alkyltransferase (ATase) activity in tumour cells. The compounds include certain pyrimidine derivatives of formula (II), wherein R is (i) a cyclic group having at least one 5- or 6-membered heterocyclic ring, optionally with a carbocyclic or heterocyclic ring fused thereto, the or each heterocyclic ring having at least one hetero atom chosen from O, N, or S, or a substituted derivative thereof; or (ii) phenyl or a substituted derivative thereof, R^2 is selected from H, C^1-C^5 alkyl, halogen or NH^2, R^4 and R^5 which are the same or different are selected from H, NH-Y' or NO_n wherein Y' is H, ribosyl, deoxyribosyl, arabinosyl, (a) wherein X is O or S, R'' is alkyl and R''' is H or alkyl, or substituted derivatives thereof, n = 1 or 2 or R^4 and R^5 together with the pyrimidine ring form a 5-or 6-membered ring structure containing one or more hetero atoms, and pharmaceutically acceptable salts thereof. They include certain guanine derivatives of formula (XIII), wherein R^6 is as defined at (i) for R above and Y' is as defined above.

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PYRIMIDINE DERIVATIVES AND GUANINE DERIVATIVES, AND THEIR USE IN TREATING TUMOUR CELLS

Technical Field

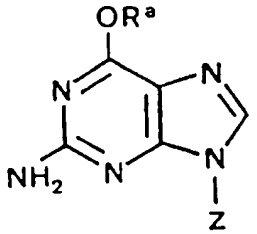
The present invention relates to pyrimidine derivatives and guanine derivatives, and their use in treating tumour cells. In particular, it relates to 6-hetarylalkyloxy pyrimidine derivatives, O^6-substituted guanine derivatives and S^6-substituted thioguanine derivatives, these compounds exhibiting the ability to deplete O^6-alkylguanine-DNA alkyltransferase (ATase) activity in tumour cells.

Background Art

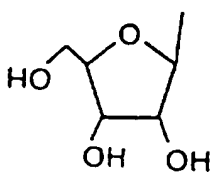
It has been suggested to use O^6-alkyl guanine derivatives possessing O^6-alkylguanine-DNA alkyltransferase depleting activity in order to enhance the effectiveness of chemotherapeutic alkylating agents, principally those that methylate or chloroethylate DNA, used for killing tumour cells. There is increasing evidence that in mammalian cells the toxic and mutagenic effects of alkylating agents are to a large extent a consequence of alkylation at the O^6-position of guanine in DNA. The repair of O^6-alkylguanine is mediated by ATase, a repair protein that acts on the O^6-alkylated guanine residues by stoichiometric transfer of the alkyl group to a cysteine residue at the active site of the repair protein in an

autoinactivating process. The importance of ATase in protecting cells against the biological effects of alkylating agents has been most clearly demonstrated by the transfer and expression of cloned ATase genes or cDNAs into ATase deficient cells: this confers resistance to a variety of agents, principally those that methylate or chloroethylate DNA. Whilst details of the mechanism of cell killing by O^6-methylguanine in ATase deficient cells is not yet clear, killing by O^6-chloroethylguanine occurs through DNA interstrand crosslink formation to a cytosine residue on the opposite strand via a cyclic ethanoguanine intermediate, a process that is prevented by ATase-mediated chloroethyl group removal or complex formation.

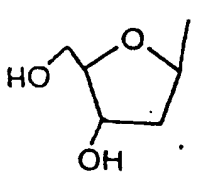
The use of O^6-methylguanine and O^6-n-butylguanine for depleting ATase activity has been investigated (Dolan et al., Cancer Res., (1986) **46**, pp. 4500; Dolan et al., Cancer Chemother. Pharmacol., (1989) **25**. pp 103. O^6-benzylguanine derivatives have been proposed for depleting ATase activity in order to render ATase expressing cells more susceptible to the cytotoxic effects of chloroethylating agents (Moschel et al., J. Med.Chem., 1992, **35** , 4486). U.S. Patent 5 091 430 and International Patent Application No. WO 91/13898 Moschel et al. disclose a method for depleting levels of O^6-alkylguanine-DNA alkyl-transferase in tumour cells in a host which comprises administering to the host an effective amount of a composition containing O^6-benzylated guanine derivatives of the following formula:



wherein Z is hydrogen, or

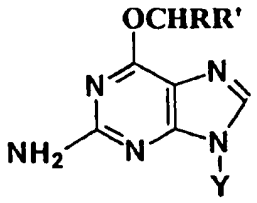


or



and R^a is a benzyl group or a substituted benzyl group. A benzyl group may be substituted at the ortho, meta or para position with a substituent group such as halogen, nitro, aryl such as phenyl or substituted phenyl, alkyl of 1-4 carbon atoms, alkoxy of 1-4 carbon atoms, alkenyl of up to 4 carbon atoms, alkynyl of up to 4 carbon atoms, amino, monoalkylamino, dialkylamino, trifluoromethyl, hydroxy, hydroxymethyl, and SO_nR^b wherein n is 0, 1, 2 or 3 and R^b is hydrogen, alkyl of 1-4 carbon atoms or aryl. Chae et al., J.Med.Chem., 1994, **37**, 342-347 describes tests on O^6-benzylguanine analogs bearing increasingly bulky substituent groups on the benzene ring or at position 9. Chae et. al., J. Med. Chem. **1995**, 38, 359-365 describe several 8-substituted O^6-benzylguanines, 2- and/or 8-substituted 6-(benzyloxy)purines, substituted 6(4)-(benzyloxy)pyrimidines, and a 6-(benzyloxy)-s-triazine which were tested for their ability to inactivate ATase . Two types of compounds were identified as being significantly more effective than O^6-benzylguanine at inactivating ATase in human HT29 colon tumour cell extracts. These were 8-substituted O^6-benzylguanines bearing electron-withdrawing groups at the 8-position (e.g. 8-aza-O^6-benzylguanine and O^6-benzyl-8-bromoguanine) and 5-substituted 2,4-diamino-6-(benzyloxy)pyrimidines bearing electron withdrawing groups at the 5-position (e.g. 2,4-diamino -6-(benzyloxy)-5-nitroso- and 2,4-diamino-6-(benzyloxy)-5-nitropyrimidine). The latter derivatives were also more effective than O^6-benzylguanine at inactivating ATase in intact HT29 colon tumour cells. WO 96/04280 published after the priority dates of this application concerns similar substituted O^6-benzylguanines and 6(4)-benzyloxypyrimidines.

The present Applicants are also Applicants in International Patent Application PCT/IE94/00031 which was published under No. WO 94/29312. WO 94/29312 (the contents of which are incorporated herein by reference in their entirety) describes O^6-substituted guanine derivatives of formula I:



(I)

wherein

Y is H, ribosyl, deoxyribosyl, or R''XCHR''', wherein X is O or S, R'' and R''' are alkyl, or substituted derivatives thereof;

R' is H, alkyl or hydroxyalkyl;

R is (i) a cyclic group having at least one 5- or 6-membered heterocyclic ring, optionally with a carbocyclic or heterocyclic ring fused thereto, the or each heterocyclic ring having at least one hetero atom chosen from O, N, or S, or a substituted derivative thereof; or

(ii) naphthyl or a substituted derivative thereof;

and pharmaceutically acceptable salts thereof.

In order to be useful for depleting ATase activity and thus enhance the effects of the above-mentioned chemotherapeutic agents, compounds should have combination of characteristics assessed by reference to:

1) *In vitro* inactivation of recombinant ATases.

2) Stability.

3) Solubility.

4) Inactivation of ATase in mammalian cells and/or tumour xenografts.

5) Sensitization of mammalian cells and/or tumour xenografts to the killing or growth inhibitory effects of the said chemotherapeutic agents

The behaviour of novel compounds in this combination of tests is unpredictable. Molecular interactions including steric factors in the unpredictability of ATase inactivation may be related to the nature of the environment of the cysteine acceptor site in the ATase molecule.

The structure of the ATase protein derived from E. coli (Ada gene) has been elucidated by X-ray crystallographic techniques (M.H. Moore et. al., EMBO Journal, 1994, **13**, 1495.). While the amino acid sequence of human ATase differs somewhat from that of bacterial origin, all known ATases (human, rodent, yeast, bacterial) contain the cysteine (Cys) acceptor site in a common fragment, Pro-Cys-His-Arg. A homology model of human ATase generated by computer from the crystal structure of the Ada protein (J.E.A. Wibley et. al., Anti-Cancer Drug Design, 1995, **10**, 75.) resembles it in having the Cys acceptor buried in a pocket deep in the protein. Considerable distortion of the structure is necessary to bring either an O^6-alkylated guanine residue in intact DNA, or even free guanine alkylated by a relatively large group like benzyl, close to the Cys acceptor. These configurational changes are initiated by a characteristic binding of duplex DNA to the protein (K. Goodtzova et. al. Biochemistry, 1994, **33**, 8385).

Since the amino acid components and dimensions of the ATase active site "pocket" are still unknown as are the details of the mechanism involved, it is impossible to predict the activity of a particular O^6-alkylated guanine or analogous ring system.

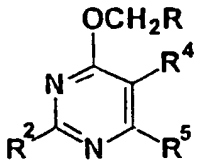
Published work in this field relates predominantly to the use of O^6-alkyl guanine derivatives having a nucleus identical to that of guanine in DNA. Chae et. al., J. Med. Chem. 1995, **38**, 359-365 have described tests on a limited number of compounds in which the

guanine ring was modified. However these compounds all had benzyl substitution at the O^6- position of the modified guanine ring or 6(4)-benzyloxy substitution on the pyrimidine ring. The observation that subtle changes in the substituents on the guanine ring or in the purine skeleton can generate agents that are very ineffective ATase inactivators, in comparison with their "parent" structure, suggests that more substantial modifications might also disrupt the ATase inactivating function.

There is a need for additional novel compounds useful for depleting ATase activity in order to enhance the effects of chemotherapeutic agents such as chloroethylating or methylating anti-tumour agents. It is a further object to provide compounds having better ATase inactivating characteristics than O^6-benzylguanine and having different solubility patterns.

Another object of the invention is to provide pharmaceutical compositions containing compounds which are useful for depleting ATase activity. A further object of the present invention is to provide a method for depleting ATase activity in tumour cells. A still further object of the invention is to provide a method for treating tumour cells in a host in such a way that they become more sensitive to the above-mentioned alkylating agents.

The present invention provides 6-hetarylalkyloxy pyrimidine derivatives of formula II:



II

wherein

R is (i) a cyclic group having at least one 5- or 6- membered heterocyclic ring, optionally with a carbocyclic or heterocyclic ring fused thereto, the or each heterocyclic ring

having at least one hetero atom chosen from O, N or S, or a substituted derivative thereof; or

(ii) phenyl or a substituted derivative thereof,

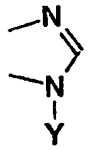
R^2 is selected from H, C_1-C_5 alkyl, halogen or NH_2,

R^4 and R^5 which are the same or different are selected from H, NH-Y' or NO_n wherein Y' is H, ribosyl, deoxyribosyl, arabinosyl, R''XCHR''' wherein X is O or S and R'' is alkyl and R''' is H or alkyl, or substituted derivatives thereof,

n = 1 or 2,

or R^4 and R^5 together with the pyrimidine ring form a 5-or 6-membered ring structure containing one or more hetero atoms, and pharmaceutically acceptable salts thereof,

with the proviso that R^2 is not NH^2 if R^4 and R^5 form a ring structure IX



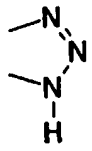
IX

wherein Y is H, ribosyl, deoxyribosyl, or R''XCHR''' wherein X is O or S, R'' and R''' are alkyl, or substituted derivatives thereof,

and with the proviso that R is not phenyl in the following circumstances a) to h):

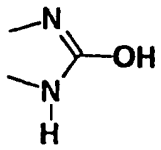
a) if R^2 and R^5 are NH_2 and R^4 is NO or NO_2

b) if R^2 is NH_2 and R^4 and R^5 form a ring structure X



X

c) if R^2 is NH_2 and R^4 and R^5 form a ring structure XI



XI

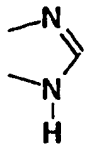
d) if R^2 is NH_2, and R^4 is NO_2 and R^5 is H or CH_3

e) if R^2, R^4 and R^5 are NH_2,

f) if R^2 and R^5 are NH_2 and R^4 is H,

g) if R^2 is H, and R^4 is NO_2 and R^5 is NH_2, or

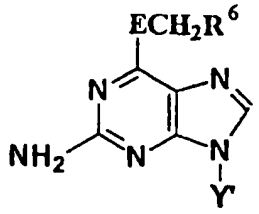
h) if R^2 is F or OH, and R^4 and R^5 form a ring structure XII



XII

Certain O^6-substituted guanine derivatives within the scope of the general formula in WO 94/29312 but not published therein have been found to have a surprisingly advantageous combination of properties which justifies the selection of such derivatives from among the class defined in WO 94/29312.

In another aspect, the present invention provides guanine derivatives of formula XIII:



XIII

wherein

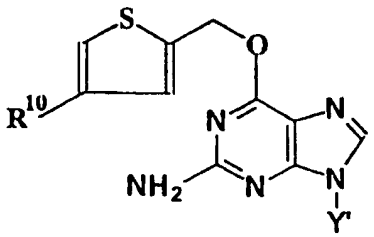
E is O or S,

Y' is as defined for formula II above,

R^6 is a cyclic group having at least one 5- or 6-membered heterocyclic ring, optionally with a carbocyclic or heterocyclic ring fused thereto, the or each heterocyclic ring having at least one hetero atom chosen from O, N or S, or a substituted derivative thereof,

and pharmaceutically acceptable salts thereof, with the proviso that compounds published in WO 94/29312 are disclaimed.

In particular, the present invention selects advantageous compounds of formula XIV:



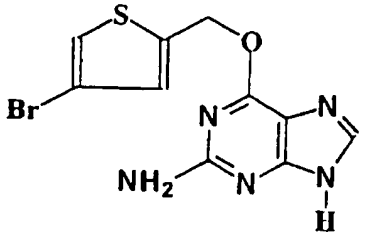
XIV

wherein

R^{10} is bromo, chloro or cyano, and

Y' is as defined for formula II.

Most preferably, R^{10} is bromo. A particularly preferred and selected compound is O^6-(4-bromothenyl)guanine having the formula XV:



XV

This compound has an advantageous combination of properties including potential for oral administration.

R or R^6 may suitably be a 5- or 6-membered heterocyclic ring or a benzo derivative thereof, in which latter case the pyrimidine moiety may be attached to R or R^6 at either the heterocyclic or the benzene ring.

In preferred embodiments, R or R^6 is a 5-membered ring containing S or O, with or without a second ring fused thereto.

Preferably, R or R^6 is a heterocyclic ring having at least one S atom; more preferably, R or R^6 is a 5-membered heterocyclic ring having at least one S atom; and most preferably, R or R^6 is a thiophene ring or a substituted derivative thereof. Alternatively, R or R^6 may be a heterocyclic ring having at least one O atom, particularly, a 5-membered heterocyclic ring having at least one O atom and more particularly R or R^6 may be a furan ring or a substituted derivative thereof. As another alternative, R or R^6 may be a heterocyclic ring having at least one N atom, particularly R or R^6 may be a 6-membered heterocyclic ring having at least one N atom and in particular, R or R^6 may be a pyridine ring.

The carbocyclic or heterocyclic ring fused to the heterocyclic ring in R or R^6 may itself be bicyclic e.g. naphthalene.

In general the term "substituted derivative" as used in relation to any of the compounds of the invention means any substituted derivative whose presence in the compound is consistent with the compound having ATase depleting activity.

In the definition of Y or Y', the term "substituted derivative" includes further substitution by one or more of the following groups: hydroxy, halo, alkoxy, amino, alkylamino, amido or ureido. In a particularly preferred group of compounds, R" is hydroxy-substituted alkyl and R''' is H, so that Y' is hydroxyalkoxymethyl, preferably having 1 to 10 carbon atoms in the alkoxy group.

In the definition of R or R^6, the term "substituted derivative" includes substitution of the heterocyclic ring(s) and/or carbocyclic ring(s) by one or more of the following groups: alkyl, alkenyl, alkynyl, alkoxy, aryl, halo, haloalkyl, nitro, cyano, azido, hydroxyalkyl, SO_nR^7 where R^7 is alkyl and n = 0,1 or 2, or a carboxyl or ester group of the formula $-COOR^8$ wherein R^8 is H or alkyl. Halo, haloalkyl, cyano, alkylenedioxy, SO_nR^7 (as defined above) and $-COOR^8$ wherein R^8 is alkyl are preferred substituents.

An alkyl, alkoxy, alkenyl, or alkynyl group preferably contains from 1 to 20, more preferably from 1 to 10 and most preferably from 1 to 5 carbon atoms. Halo includes iodo, bromo, chloro or fluoro. An aryl group preferably contains from 1 to 20, more preferably from 1 to 10 carbon atoms, particularly 5 or 6 carbon atoms.

One embodiment of the invention provides a pharmaceutical composition containing compounds of formula II or formula XIII, as defined above, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable excipient. Optionally the composition may also contain an alkylating agent such as a chloroethylating or methylating agent.

In a further embodiment, the present invention provides a method for depleting ATase activity in a host comprising administering to the host an effective amount of a composition containing a compound of formula II or formula XIII as defined above, or a pharmaceutically acceptable salt thereof, more particularly a pharmaceutical composition as defined above. This method may alternatively be defined as a method of depleting ATase mediated DNA repair activity in a host.

The invention further provides a method for treating tumour cells in a host comprising administering to the host an effective amount of a composition containing a compound of formula II or

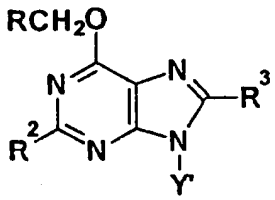
formula XIII as defined above or a pharmaceutically acceptable salt thereof, more particularly a pharmaceutical composition as defined above and administering to the host an effective amount of a composition containing an alkylating agent. The method may be used for treatment of neoplasms including those which are known to be sensitive to the action of alkylating agents e.g. melanoma and glioma and others whose resistance to treatment with alkylating agents alone may be overcome by the use of an inactivator according to the invention.

The term "pharmaceutically acceptable salts" as used in this description and the claims means salts of the kind known in the pharmaceutical industry including salts with inorganic acids such as sulfuric, hydrobromic, nitric, phosphoric or hydrochloric acid and salts with organic acids such as acetic, citric, maleic, fumaric, benzoic, succinic, tartaric, propionic, hexanoic, heptanoic, cyclopentanepropionic, glycolic, pyruvic, lactic, malonic, malic, o-(4-hydroxy-benzoyl)benzoic, cinnamic, mandelic, methanesulfonic, ethanesulfonic, 1,2-ethanedisulfonic, 2-hydroxyethanesulfonic, benzenesulfonic, p-chlorobenzenesulfonic 2-naphthalenesulfonic, p-toluenesulfonic, camphorsulfonic, 4-methyl-bicyclo[2.2.2]oct-2-ene-1-carboxylic, glucoheptonic, 4,4'-methylenebis(3-hydroxy-2-naphthoic), 3-phenylpropionic, trimethyl-acetic, tertiary butylacetic, lauryl sulfuric, gluconic , glutamic, hydroxynaphthoic, salicylic, stearic, or muconic, and the like.

Subject to the provisos above the preferred compounds of the invention are those of:

Type 1

Formula III



III

wherein:

R is as defined for formula II, particularly furyl or thienyl unsubstituted or substituted, preferably with a halogen such as chlorine, bromine or fluorine, or with cyano

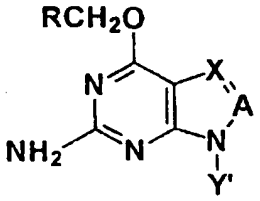
Y' is as defined for formula XIII, preferably Y' is H or $HOCH_2CH_2OCH_2$-;

R^2 is H, NH_2, C_1-C_5 alkyl, preferably methyl, or halogen, preferably fluorine;

R^3 is H or OH;

Type 2

Formula IV



IV

wherein:

R is as defined for formula II, particularly phenyl, thienyl or furyl unsubstituted or substituted preferably with a halogen such as chlorine, bromine or fluorine, or with cyano, or phenyl having a methylenedioxy ring structure fused thereto;

Y' is as defined for formula XIII;

X is CH or N;

A is CH or N; and preferably when X = N, A = CH

Formula V

wherein:

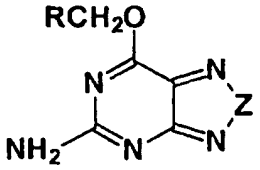
R is as defined for formula II

X is CH or N

A is CH or N;

Type 3

Formula VI



VI

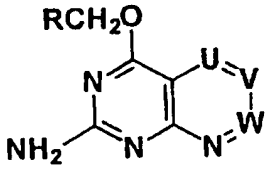
wherein:

R is as defined for formula II, particularly, thienyl or furyl unsubstituted or substituted preferably with a halogen such as chlorine or bromine;

Z is O or S or CH = CH;

A particularly preferred group of compounds of this type are Q^6-(4-halothenyl)-8-thiaguanines, particularly Q^6-(4-bromothenyl)-8-thiaguanine.

Formula VII



VII

wherein:

R is as defined for formula II;

U is CH or N;

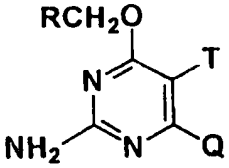
V is CH or N;

W is CH or N;

provided that U, V and W are not all CH.

Type 4

Formula VIII



VIII

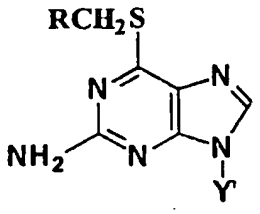
wherein:

R is as defined for formula II, particularly thenyl or furyl optionally substituted with halogen preferably one or more of chlorine, bromine or fluorine;

T is H, NH_2 or NO_n where n = 1 or 2;
Q is H, NH_2 or NO_n where n = 1 or 2;

Type 5

Formula XVI



XVI

wherein

R is as defined for formula XIII

Y' is as defined for formula II

Brief Description of Drawings

The invention will be described in greater detail with reference to the accompanying drawings, in which:

Figures 1 to 4 are graphs showing the effect of pretreatment with compound B.4316 on Raji cell sensitization to different chemotherapeutic agents. Each graph plots percentage growth against the concentration (µg/ml) of the chemotherapeutic agent in the presence and absence of B.4316.

Figure 1 shows the effect of 1uM B.4316 pretreatment on Raji cell sensitization to temozolomide.

Figure 2 shows the effect of 10μm B.4316 pretreatment on Raji cell sensitization to BCNU.

Figure 3 shows the effect of 10μM B.4316 pretreatment on Raji cell sensitization to fotemustine.

Figure 4 shows the effect of 10μM B4316 pretreatment on Raji cell sensitization to melphalan and cisplatin.

Figure 5 is a histogram showing the effect of 10μM B4316 pretreatment on Raji cell sensitization to different chemotherapeutic agents, measured as sensitization factor (SF, defined below) based on D_{50} except for fotemustine where SF is based on D_{80}.

Figure 6 is a similar histogram showing the effect of 10μM B4349 pretreatment on Raji cell sensitization to different chemotherapeutic agents, with SF as for Figure 5.

Figure 7 is a series of histograms showing the inactivation of ATase in A375M tumours and murine host tissues two hours after interperitoneal (i.p.) administration of various inactivator compounds at 5mg/kg. Inactivation was calculated as % of control ATase activity, measured as fm/mg protein.

Figure 8 is a graph showing the kinetics of ATase depletion and recovery in A375M tumours and murine host tissues after administration of B.4363 (20 mg/kg i.p.). The graph plots % of control ATase activity against time (hours).

Figure 9 is a graph of percentage residual activity of pure recombinant human ATase following incubation with increasing concentrations of inactivators O^6-benzylguanine (BeG), O^6-thenylguanine (B.4205) and O^6-(4-bromothenyl)guanine (B.4280). The line at 50% residual activity is used for calulating I_{50} values i.e. the concentration of inactivator required to

produce a 50% reduction in ATase activity. The I_{50} values shown are extrapolated from the curves. Preincubation was for 1 hour after which [^{3}H]-methylated substrate was added to determine residual activity of ATase.

Figure 10A is three graphs of percentage cell growth against temozolomide concentration (μg/ml) showing the effect of pretreatment with BeG, B.4205 and B.4280 (0.5μM final concentration) on the sensitivity of Raji cells to the growth inhibitory effects of temozolomide. Inactivator or vehicle was given 2 hours prior to temozolomide.

Figure 10B is a histogram for the inactivators of Figure 10A showing the sensitization factor based on D_{50} of Raji cells to growth inhibition by temozolomide.

Figure 11 is a histogram of ATase activity (fm/mg) against time (hours) showing the effect of ATase inactivators BeG, B.4205 and B.4280 on ATase activity in human melanoma xenografts grown in nude mice. Animals were given a single dose of the inactivators intraperitoneally (i.p.) at 30mg/kg or 60mg/kg and sacrificed after the times shown.

Figure 12 is a histogram showing the effect of ATase inactivators on ATase activity (fm/mg) in human melanoma xenografts grown in nude mice. Animals were given B.4205 or temozolomide alone or B.4205 or B.4280 in combination with temozolomide (50mg/kg)i.p. at the doses shown on three consecutive days (except where indicated) and sacrificed 24 hours after the final dose. The vehicles were corn oil for the inactivators and PBS (20%DMSO) for temozolomide.

Figure 13 is a histogram showing the effect of ATase inactivators on ATase activity in livers of nude mice. Animals were given the B.4205 or temozolomide alone or B.4205 or B.4280 in combination with temozolomide (50mg/kg, i.p.) at the doses shown on

three consecutive days (except where indicated) and sacrificed 24 hours after the final dose.

Figure 14A is a graph of % tumour growth against time (days) showing the effect of B.4205 on the sensitivity of human melanoma xenografts to growth inhibition by temozolomide. Animals were untreated, given temozolomide alone (100mg/kg, i.p.) or B.4205 (5, 10 or 20mg/kg i.p.) followed 1 hour later by temozolomide (100mg/kg, i.p.) on five consecutive days. Tumour growth was monitored as described. The data from a number of separate studies are presented.

Figure 14B is a graph of number of surviving mice against time (days) showing survival of animals (tumour-bearing nude mice) used in the study shown in Figure 14A. Groups of animals in which the xenografts had reached the maximum size were terminated.

Figure 15A is a graph showing the effect of B.4280 on the sensitivity of human melanoma xenografts to growth inhibition by temozolomide. Animals were untreated, given temozolomide alone (100mg/kg. i.p.) or B.4280 alone (20mg/kg, i.p.) or B.4280 (1, 5, 10 or 20 mg/kg, i.p.) followed 1 hour later by temozolomide (100mg/kg, i.p.) on five consecutive days. Tumour growth was monitored as described. The data from a number of separate studies are presented.

Figure 15B is a graph showing the survival of the animals (tumour-bearing nude mice) used in the study shown in Figure 15A. Groups of animals in which the xenografts had reached the maximum size were terminated.

Figure 16A is a graph of % tumour growth against time (days) showing the comparison of the effect of B.4280 given i.p. and orally (p.o.) on the sensitivity of human melanoma xenografts to growth inhibition by temozolomide. Animals were untreated, given temozolomide alone (100mg/kg) or B.4280 alone (20mg/kg, i.p.) or B.4280 (20mg/kg, i.p.) or B.4280 (30mg/kg, p.o.) followed 1 hour later by temozolomide (100mg/kg, i.p.) on five consecutive days.

Tumour growth was monitored as described. The data from a number of separate studies are presented.

Figure 16B is a graph showing the survival of the animals used in the study shown in Figure 16A. Groups of animals in which the xenografts had reached the maximum size were terminated.

Figure 17 is a graph showing the survival of animals in a comparative test of the effects of BeG, B.4205 and B.4280 in combination with temozolomide (TZ) in non-tumour-bearing DBA_2 mice. Animals were given temozolomide alone (100mg/kg i.p) or BeG (10 or 20mg/kg i.p.), B.4205 (10 or 20 mg/kg i.p.) or B.4280 (10 or 20 mg/kg i.p.) followed one hour later by temozolomide (100mg/kg i.p.) on five consecutive days.

Figures 18 to 21 consist of pairs of graphs showing the kinetics of ATase depletion and recovery in various tumours and murine host tissues after administration of B.4280 at the doses indicated. The graphs plot ATase activity (fm/mg protein) and % of control ATase activity against time (hours):

Figure 18 relates to B.4280 (20 mg/kg i.p.) in A375M tumours and other tissues.

Figure 19 relates to B.4280 (30mg/kg p.o.) in A375M tumours and other tissues

Figure 20 relates to B.4280 (30mg/kg i.p.) in MCF-7 tumours and other tissues.

Figure 21 relates to B.4280 (20mg/kg i.p.) in DU-145 tumours and other tissues.

Figure 22 is a graph of % tumour growth against time (days) showing the effect of B.4280 on the sensitivity of MCF-7 tumours to growth inhibition by temozolomide. Animals were untreated, were

given temozolomide alone (100 mg/kg, i.p.) or B.4280 (PaTrin-2) (20mg/kg i.p.) alone, or B.4280 (20mg/kg i.p.) followed 1 hour later by temozolomide (100mg/kg i.p.) on five consecutive days.

Figure 23 consists of graphs of % tumour growth, number of surviving mice and mean weight (g) against time (days) showing the effect of a single dose of B.4280 (PaTrin-2) on the sensitivity of melanoma tumours to growth inhibition by a single dose of fotemustine. Animals were given fotemustine (20mg/kg i.p.) alone, or B.4280 (30mg/kg p.o) followed 1 hour later by fotemustine (20mg/kg i.p.).

Figure 24 consists of graphs of % tumour growth and number of surviving mice against time (days) for sensitization of A375M tumours with B.4205 (PaTrin-1) and B.4280 20mg/kg pretreatment followed by 150mg/kg temozolomide using a 5 day schedule as for Figure 22.

Figure 25 consists of graphs of % tumour growth, number of surviving mice and mean weight (g) against time showing sensitization of A375M tumours to temozolomide (100mg/kg i.p.) following administration of 20mg/kg B.4349 or B.4351 (i.p.).

Figure 26 is a figure showing ATase activities in A375M tumours and murine host tissues at 2 hours and 24 hours following i.p. administration of 90mg/kg B.4335.

In the specification the abbreviations "1h" or "2h" etc. mean "1 hour", "2 hours" etc.. In the drawings the abbreviations "Temo" and "Tz" refer to temozolomide.

Figure 27 consists of graphs of % tumour growth and weight (% of day 1 value) against time (days) showing tumour DU-145 prostate xenograft growth after temozolomide (100mg/kg/day) and/or B.4280 (PaTrin-2)(20mg/kg/day) days 1-5. Points are the means of values from at least 4 mice. Growth delays in each group were (p value):

PaTrin-2 alone 0.1 days (>.05); temozolomide alone 7.8 (>.05). Both agents 15.3 (0238).

Figure 28 is a reaction scheme for synthesis of O^6[^{3}H]-(4-bromothenyl)guanine.

Figure 29 shows co-chromatography of authentic B.4280 and readioactivity in the product of O^6-[^{3}H]-(4-bromothenyl)guanine synthesis. Shading indicates counts recovered (LH axis) and the line OD at 254nm (RH axis).

Figure 30 shows transfer of radioactivity from O^6-[^{3}H]-(4-bromothenyl)guanine to rhATase after one hour incubation at 37°C.

Description of the Preferred Embodiments

Examples of compounds of the invention are shown in Tables 1a and 1b. They were synthesized by the procedures presented below, adapted as appropriate.

Type 1

A. O^6-Substituted hypoxanthines were made by the action of alkoxide RCH_2ONa on the quaternary salt N,N,N-trimethyl-1H-purin-6-aminium chloride.[1]

B. O^6-Substituted 2-methylhypoxanthines were made similarly, from the quaternary salt from diazabicyclooctane (DABCO) and 6-chloro-2-methylpurine.[2]

C. O^6-Substituted 2-fluorohypoxanthines were made by diazotisation of the corresponding guanines using sodium nitrite and concentrated fluoboric acid at -25°C.[3]

D. O^6-Substituted 9-(2-hydroxyethoxymethyl)guanines were made by condensing the corresponding guanines after silylation with 2-acetoxyethoxymethyl bromide in the presence of mercuric cyanide followed by saponification of the O-acetyl group.[4]

E. O^6-Substituted 8-hydroxyguanines were made from 6-hetarylmethyl-2,4,5-triaminopyrimidines and 1, 1-carbonyldiimidazole in DMF.[5] Reaction of 6-chloro-2,4-diaminopyrimidine with alkoxide in DMSO, followed by nitrosation with sodium nitrite in aqueous acetic acid and reduction using sodium hydrosulphite in aqueous DMF, gave the 2, 4, 5-triamines.

Type 2

A. O^6-Substituted 8-azaguanines were made from the above triamines and sodium nitrite in aqueous acetic acid.[6]

B. O^6-Substituted 8-aza-7-deazaguanines were made from the alkoxide RCH_2ONa and 2-amino-6-chloro-8-aza-7-deazapurine[7] in sulfolane or from the DABCO quaternary salt (in DMSO solvent) derived from it.

Type 3

A. O^6-Substituted 8-oxaguanines were made by lead tetraacetate oxidation[8] of 6-hetarylmethyl-2,4-diamino-5-nitrosopyrimidines obtained as under Type IE.

B. O^6-Substituted 8-thiaguanines were made from the triamine intermediates under Type IE and N-tosylthionylimine in pyridine.[9]

C. O^4-Substituted pterins were made from these triamines and glyoxal with sodium metabisulphite.[10]

Type 4

A and B.

These pyrimidines were obtained as under Type IE.

C. $\underline{O}^6$-Substituted 2,4-diamino-5-nitropyrimidines were made by the action of alkoxide RCH_2ONa in DMSO on 6-chloro-2,4-diamino -5-nitropyrimidine. [11]

Type 5

$\underline{S}^6$-Substituted 6-thioguanines were prepared from the thiolate RCH_2SNa and the quaternary salt 2-amino-$\underline{N}$,$\underline{N}$,$\underline{N}$-trimethyl-1 $\underline{H}$-purin-6-aminium chloride (WO 94/29312).

$\underline{O}^6$-Substituted guanines as listed in Tables 6a and 6b were made by the standard preparation as described in WO 94/29312, usually with 3mmol alcohol RCH_2OH per mmol quaternary salt.

The alcohols were made as described in WO 94/29312 by sodium borohydride reduction of the corresponding aldehydes, with two exceptions. For 4-bromothenyl alcohol[12] required for B.4280 the aldehyde is commercially available. 5-Chlorothiophen-2-aldehyde[13] and 5-methylthiothiophen-2-aldehyde[14] were prepared by Vilsmeier reaction on 2-chlorothiophen and 2-methylthiothiophen respectively. Sodium borohydride reduction of the methylthioaldehyde followed by sodium periodate oxidation[15] of the resulting methylthioalcohol yielded the methylsulphinylalcohol required for B.4294. Reduction of the chloroaldehyde gave 5-chlorothenyl alcohol[16] for B.4281.

Several other aldehydes were obtained by halogenation of the appropriate thiophen aldehyde or furfural. Thus, direct bromination gave 5-bromofurfural[17] and thence the alcohol[18] for B.4336. Halogen in presence of aluminium chloride on thiophen-2-aldehyde yielded 4-chlorothiophen-2-aldehyde[19] (for the alcohol for

B.4298), on thiophen-3-aldehyde yielded 2-bromothiophen-4-aldehyde[20] (and eventually B.4313), and on 5-chlorothiophen-2-aldehyde yielded 4,5-dichlorothiophen-2-aldehyde[21] (for the alcohol[22] for B.4318).

Cyanoaldehydes were obtained from copper cyanide and the corresponding bromoaldehydes in refluxing dimethylformamide. 5-Cyanothiophen-2-aldehyde[23] and its 4-cyano isomer[24] then gave the 5-cyano and 4-cyano[25] alcohols, for B.4283 and B.4317 respectively.

4-Methoxythenyl alcohol[26] (for B.4300) was prepared as described from 2,3-dibromosuccinic acid and methyl thioglycollate, and ultimate reduction of the methyl ester (not aldehyde in this case) by lithium aluminium hydride and 2-chloro-4-picolyl alcohol[27] (for B.4321) by sodium borohydride reduction[28] of the corresponding acid chloride, made in turn from reaction[29] of phosphorus oxychloride/pentachloride on isonicotinic acid N-oxide.

For B.4282, 3-pyridinemethanol N-oxide is commercially available. 5-Methylsulphonylthenyl alcohol (for B.4309) was obtained by m-chloroperbenzoic acid (MCPBA) oxidation of the alcohol resulting from reduction of 5-methylthio-2-thiophenecarboxaldehyde [30].

6-Chloro-3-pyridinemethanol (for B.4319) and 5-bromo-3-pyridinemethanol (for B.4320) were made by treatment of 6-chloro and 5-bromonicotinic acids respectively with phosphorus oxychloride/pentachloride and reduction of the resulting acid chlorides with sodium borohydride[28]. Isothiazole-4-methanol (for B.4354) was obtained by reduction of the corresponding methyl ester (A. Adams and R. Slack, J. Chem. Soc. 1959, 3061) with lithium aluminium hydride (M. Hatanaka and T. Ishimaru, J. Med. Chem. 16, 1973, 978).

4-bromo-2-thiophenecarboxaldehyde was converted into the

4-lithio derivative (A.L. Johnson, J. Org. Chem, 41, 1976, 1320) of its ethylene acetal and reaction of this organometallic with dimethyl disulphide followed by acid hydrolysis gave 4-methylthio-2-thiophenecarboxaldehyde (R. Noto, L. Lamartina, C. Arnone and D. Spinelli, J. Chem. Soc., Perkin Trans. 2, 1987, 689). Sodium borohydride reduced this aldehyde to the 4-methylthio alcohol (for B.4356), which in turn with one of two equivalents of MCPBA yielded the 4-methylsulphinyl and 4-methylsulphonyl alcohols (for B.4377 and B.4361 respectively). Reaction of the above organometallic with naphthalene-2-sulphonyl azide (A.B. Khare and C.E. McKenna, Synthesis, 1991, 405) and sodium pyrophosphate followed by hydrolysis by the method (P. Spagnolo and P. Zanirato, J. Org. Chem., 43, 1978, 3539) for the preparation of other azidothiophene aldehydes gave 4-azido-2-thiophenecarboxaldehyde leading to the alcohol for B.4373.

5-Iodo-3-thiophenemethanol (for B.4357) came from the aldehyde obtained by treatment of 3-thiophenecarboxaldehyde with iodine-iodic acid-sulphuric acid (R. Guilard, P. Fournari and M. Person, Bull. Soc. Chim. France, 1967, 4121).

2-Naphtho[2, 1-b]thienylmethanol (for B.4366) was prepared by lithium aluminium hydride reduction of the corresponding carboxylic acid (M.L. Tedjamulia, Y. Tominaga, R.N. Castle and M.L. Lee, J. Heterocycl. Chem., 20, 1983, 1143). 5-Phenylthenyl alcohol (m.p. 91.5^{o}C, for B.4378) resulted from sodium borohydride reduction of the aldehyde (P. Demerseman, N.P. Buu-Hoi and R. Royer, J. Chem. Soc., 1954, 4193) obtained by Vilsmeier reaction of 2-phenylthiophene (from Gomberg-Bachmann reaction (N.P. Buu-Hoi and N. Hoan, Rec. trav. chim., 69, 1950, 1455) of benzenediazonium chloride and alkali with thiophene).

By way of specific example, the preparation of O^6-(4-bromothenyl)guanine (B.4280) will now be described.

Preparation of O^6-(4-bromothenyl)guanine
A solution of 4-bromothenyl alcohol[12] [4.63g, 24mmol; R_f0.38 in TLC(PhMe-MeOH, 4:1)] in DMSO (4ml) was treated cautiously with sodium hydride (60% in oil; 0.64g, 16mmol). After 1 hour's stirring, 2-amino-N,N,N-trimethyl-1H-purin-6-aminium chloride (1.83g, 8mmol) was added. After 1 hour's further stirring, acetic acid (1.3ml) followed by ether (240ml) was added and the solid filtered off after 1-2h. Removal of solvents and excess of alcohol (b.p. 85-90°C/0.4mm) from the filtrate yielded a negligible second fraction (17mg). The main crop was triturated with water (10ml), affording substantially pure product (1.89g, 73%) with R_f 0.22 in TLC (PhMe-MeOH, 4:1). It was recrystallized by dissolving in hot methanol (100ml) and then concentrating. Analytical data are given in Tables 6a and 6b, together with data for other compounds. Other typical synthetic procedures are described by way of example in a special section later in this text.

Compounds of formula II or XIII in which Y' is R''XCHR''' and R''' is alkyl (seco-nucleosides) may be prepared by an analogous preparation to the reaction of O^6-benzylguanine with -chloro-ethers (MacCoss et al., Tetrahedron Lett.; European Patent Application No. 184,473., loc. cit.) or with alkyl bromides (e.g. Kjellberg, Liljenberg and Johansson, Tetrahedron Lett., 1986, 27, 877; Moschel, McDougall, Dolan, Stine, and Pegg, J.Med. Chem., 1992, 35, 4486).

Typical "sugar" components corresponding to R''XCHR''', leading to seco-nucleosides, are made by methods described in e.g. McCormick and McElhinney, J. Chem. Soc., Perkin Trans. 1, 1985, 93; Lucey, McCormick and McElhinney, J. Chem. Soc. Perkin Trans. 1, 1990, 795.

Compounds of formula II or XIII in which Y is ribosyl or deoxyribosyl (nucleosides) may be prepared by methods analogous to the syntheses of O^6-benzylguanine riboside and 2-deoxyriboside (Moschel et al. 1992; cf. Gao, Fathi, Gaffney et al., J. Org. Chem.,

1992, **57**, 6954; Moschel, Hudgins and Dipple, J. Amer. Chem. Soc., 1981, **103**, 5489) (see preparation of Ribosides above).

Industrial Applicability

The amount of the compound of the present invention to be used varies according to the effective amount required for treating tumour cells. A suitable dosage is that which will result in a concentration of the compound of the invention in the tumor cells to be treated which results in the depletion of ATase activity, e.g. about 1 - 2000 mg/kg body weight, and preferably 1 - 800 mg/kg body weight, particularly 1-120 mg/kg body weight, prior to chemotherapy with an appropriate alkylating agent.

The pharmaceutical composition of the invention may be formulated in conventional forms with conventional excipients, as described for example in WO 91/13898 and WO 96/04281 and U.S. Patents 5,091,430 and 5,352,669, the contents of which are incorporated herein by reference in their entirety. The composition may contain the inactivator according to the invention together with an appropriate alkylating agent; or the composition may comprise two parts, one containing the inactivator and the other containing the alkylating agent. The method of administering the compounds of theinvention to a host may also be a conventional method, as described in WO 91/13898 for example. For administration of an inactivator according to the invention to patients, the pharmaceutical composition may suitably contain the inactivator in a suitable vehicle such as 40% polyethyleneglycol 400 in saline solution, or in saline or 3% ethanol (in saline), for intravenous injection, or in a powder form in suitable capsules for oral administration.

Alkylating agents may be administered in accordance with known techniques and in conventional forms of administration, as described in WO 91/13898 for example or preferably as a single dose immediately after or up to 24 hours after but preferably around 2

hours after administration of the ATase inactivating agents and also at doses lower than those used in standard treatment regimen. A reduction in dose may be necessary because the inactivators would generally be anticipated to increase the toxicity of the alkylating agents. Examples of chloroethylating agents include 1,3 bis (2-chloroethyl)-1-nitrosourea (BCNU), 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU), fotemustine, mitozolomide and clomesone and those described in McCormick, McElhinney, McMurry and Maxwell J. Chem. Soc. Perkin Trans. I, 1991, 877 and Bibby, Double, McCormick, McElhinney, Radacic, Pratesi and Dumont Anti-Cancer Drug Design, 1993, 8, 115. Examples of methylating agents include temozolomide (British Patent GB 2 104, 522 and U.S. Patent 5,260,291 the contents of which are incorporated herein in their entirety) and dacarbazine, procarbazine, and streptozocin.

Methods

O^6-alkylguanine-DNA-alkyltransferase assay

Varying amounts of recombinant ATase or cell/tissue extracts were incubated with [^{3}H]-methylnitrosourea-methylated calf thymus DNA (specific activity, 17Ci/mmol) at 37°C for 1 hour in a total volume of 300µl buffer I/[50mM Tris/HCl (pH8.3), 3mM dithiothreitol (DTT), 1mM EDTA] containing 1mg/ml bovine serum albumin (IBSA) for recombinant ATases and tissue extracts, or 1.1ml buffer I for cell extracts. After incubation, bovine serum albumin (100µl of 10mg/ml in buffer I) and perchloric acid (100µl of 4M perchloric acid for 300µl volumes and 400µl for 1.1ml volumes) and 2ml of 1M perchloric acid were added. Samples were then heated at 75°C for 50 minutes to hydrolyze the DNA. Samples were then centrifuged at 3,000rpm for 10 minutes and the precipitate washed once with 4ml of 1M perchloric acid, before being resuspended in 300µl of 0.01M sodium hydroxide and dissolved in 3ml of aqueous scintillation fluid (Ecoscint A, National Diagnostics). Counting efficiency was approximately 30%. ATase specific activity was calculated from the region where the

activity was proportional to the amount of extract added, since with higher amounts of extracts the reaction becomes substrate limiting. ATase activity is expressed as fmol methyl transferred to protein per mg of total protein in the extract.

Method of Purification of Recombinant ATases

The cDNA cloning and overexpression of the human ATase has been reported previously[30]. Purification of the recombinant proteins was achieved either by affinity chromatography through a DNA-cellulose column as described by Wilkinson et al.,[31, 32], or by DEAE-cellulose ion-exchange chromatography. For the latter, the ATase protein was partially purified by ammonium sulphate precipitation (30 - 60%) and dialyzed against 10 mM Tris-HCl pH 7.5, 1 mM DTT, 2 mM EDTA, 10% glycerol, before loading on a DEAE-cellulose column. The ATase was then eluted with a 0-0.1 M NaCl gradient. The purified human ATase protein retained activity for more than one year when stored at high concentration at -20°C in buffer I [50 mM-Tris/HCl (pH 8.3)/3 mM-dithiothreitol/1 mM-EDTA] and could be thawed and refrozen several times without substantial loss of activity.

Incubation with Inactivators and ATase assay

Compounds to be tested were dissolved in DMSO to a final concentration of 10 mM and diluted just before use in buffer I . Recombinant ATase was diluted in buffer I containing 1 mg/ml bovine serum albumin (IBSA) and titrated as described above in order that the reaction be conducted under ATase, and not substrate, limiting conditions. In each assay, fixed amounts of ATase (60-75 fmol) were incubated with varying amounts of O^6-benzylguanine, or test compound in a total volume of 200 µl of IBSA containing 10 µg of calf thymus DNA at 37°C for 1 hour. The [^{3}H]-methylated-DNA substrate (100 µl containing 4 µg of DNA and 100 fmol of O^6-methylguanine) was then added and incubation continued at 37° for 1 hour, until the reaction was complete. Following acid

hydrolysis of the DNA as described above the [^{3}H]-methylated protein was recovered and quantitated by liquid scintillation counting. I_{50} is the concentration of inactivator required to produce a 50% reduction in ATase activity under the above conditions.

Cell Culture and preparation of extracts

Mammalian cells including Raji cells (a human lymphoblastoid cell line from a Burkitt's lymphoma), A375M cells (human melanoma cells), MCF-7 cells (human breast cancer cells) and PC3 and DU145 (both human prostate cancer cells) were cultured under standard conditions. For example, Raji cells were grown in suspension culture in RPMI medium supplemented with 10% horse serum. Cell pellets were resuspended in cold (4°C) buffer I containing 2 μg/ml leupeptin and sonicated for 10 seconds at 12 μm peak to peak distance. After cooling in ice, the cells were sonicated for a further 10 seconds at 18 μm. Immediately after sonication, 10 μl/ml of phenylmethanesulphonylfluoride (PMSF 8.7 mg/ml in 100% ethanol) was added and the sonicates centrifuged at 15 000cpm for 10 minutes at 4°C to pellet cell debris. The supernatant was transferred to a tube on ice and kept for determination of ATase activity (see above).

Stability of Inactivators at 37°C by Spectrophotometry.

Inactivators (10mM in DMSO) were diluted to 0.1mM in prewarmed degassed PBS (pH 7-7.2). PBS (Phosphate buffered saline) is 0.8% NaCl, 0.02% KCl, 0.15% $Na_2H_2PO_4$, 0.02% KH_2PO_4. Samples were immediately transferred to a CARY13 spectrophotometer (cuvette block held at 37°C) and scanned at an appropriate wavelength (according to the spectral properties of the compound) at 5-10 minute intervals for up to 80 hours. The results were expressed as percentage absorbance change versus time and T1/2 values (half life) extrapolated from this. In the tables the results of these tests are identified by "in PBS" or "by Spec".

Stability of Inactivators by ATase Assay

Inactivators (10μM in DMSO) were diluted to the appropriate concentration (I_{90} calculated from previous I_{50} determination

data) in buffer I without DTT and incubated for varying times at 37°C. Samples were then taken for use in the competition assay to assess the compound's ability to inactivate human ATase. The results were expressed as reduction in inactivating activity versus time and T 1/2 values extrapolated from this.

Inactivation of ATase activity in Raji cells.

Raji cells were diluted to between 5 x 10^5/ml and 10 ^{6}/ml in medium containing either the appropriate concentration of inactivator or an equivalent volume of vehicle (DMSO). Following incubation at 37°C for 2 hours the cells were harvested by centrifugation, washed twice with PBS and the resulting cell pellets (between 5 x 10^6 and 10^7 cells per pellet) stored at -20°C. ATase activity was determined as described above, in duplicate cell extracts and expressed as the percentage activity remaining, based on that present in the untreated controls (350-450 fm/mg depending on the assay). I_{50} (i.e concentration of inactivator required to reduce ATase activity by 50%) values were extrapolated from this data.

Sensitization of Mammalian cells to Cytotoxic Agents.

Sensitization of mammalian cells to the cytotoxic effects of BCNU, temozolomide and other cytotoxic agents following a 2 hour pretreatment with inactivator was analysed using an XTT-based growth inhibition assay[22]. Cells were plated in 96 well plates (for example in the case of Raji cells at 500 cells/well) and incubated at 37°C for 30 minutes prior to the addition of medium containing either the appropriate concentration of inactivator or an equivalent volume of vehicle. Following a 2 hour incubation at 37°C, medium containing either increasing doses of cytotoxic agent or equivalent vehicle was added and the cells allowed to grow for 6 days. At this time XTT solution was added and the cells incubated for a further 4 hours at 37°C. The resulting red/orange formazan reaction product was quantified by measuring absorption at 450nm on a microtitre platereader.

From this data the percentage growth of cells relative to that in control wells was determined for a range of BCNU, temozolomide or other cytotoxic agent doses in both the presence and absence of inactivator. Sensitization factor (SF) based on D_{50} ($D_{50}.^{C}/D_{50}.^{I}$) was determined by dividing the D_{50} (i.e. dose at which there was 50% growth versus the controls untreated with alkylating agent) calculated for the cytotoxic agent alone ($D_{50}.^{C}$) by that for the cytotoxic agent plus inactivator ($D_{50}.^{I}$). A value of one (1) thus indicates no sensitization by the inactivator. Comparable Sensitization factors were also determined in some cases based on D_{60} and D_{80}, i.e. the dose at which there was respectively 60% or 80% growth compared to the untreated controls. In Table 3 the Sensitization Factor $D_{50}.^{C}/D_{50}.^{I}$ is shown as D_{50} control / D_{50} 'B', with the letter 'B' referring to the inactivator compound.

<u>Xenograft Studies</u>

Animals

Swiss mouse derived athymic male mice (o/nu) weighing between 20-30g were obtained from ZENECA Pharmaceuticals, Alderley Park, Macclesfield, Cheshire, SK10 4T6, England. Animals were housed 4-5/cage in filter top cages and had access to food and water *ad libitum*. All animals were maintained under a controlled 12h-light-12h-dark cycle. These animals were used for all tests except those which are shown in Figures 11 and 17 and Table 8, as mentioned below.

Cells

A375M (human melanoma) and DU145 (human prostate cancer) cells were grown in DMEM containing 10% foetal bovine serum (FBS). MCF-7 (human breast cancer cells) were grown in DMEM containing 10% FBS supplemented with 100iu insulin.

Tumours

A375M, DU145 and MCF-7 cells (10^6) in 100μl PBS were

injected subcutaneously into the right-hand flank of 8-10 week old o/nu athymic mice. These cells were allowed to develop into a tumour for 3-4 weeks (A375M and DU145 cells) and 4-6 weeks (MCF-7 cells). Once established, tumours were maintained by subcutaneous implantation of $2mm^3$ blocks into the right-hand flank of athymic o/nu mice. MCF-7 tumours are oestrogen postive and require oestrogen for growth. This was supplied as a subcutaneous implant (see below) at the tail base simultaneously to the tumour implant and monthly thereafter.

Preparation of Oestrogen Pellets

468mg β-oestradiol was added to 9.7g silastic and mixed until evenly distributed. 1.1g of curing agent was added and the whole mixture spread into 3 (26mm x 12mm x 1mm) glass fomers. These were then incubated at 42°C overnight before being cut into 2mm x 2mm x 1mm cubes, so that each pellet contained 2mg estradiol.

ATase Depletion Experiments

Tumours were implanted as previously described and left 3-6 weeks to establish depending on tumour type. An inactivator was homogenized in corn oil at 5mg/ml before administration by interperitoneal injection (i.p.) or oral gavage (p.o.). Mice were sacrificed at various times up to 72h and tumours and murine tissues taken for ATase assay. Samples were snap frozen and stored at -20°C until analysis.

Tumour Sensitization Experiments

O/nu mice were treated with the appropriate dose of the inactivator as indicated (4mg/ml in corn oil) or the appropriate vehicle as a control 1 hour prior to administration of the appropriate dose of the cytotoxic agent (e.g.temozolomide 6mg/ml in PBS + 20% DMSO) or fotemustine or BCNU (2mg/ml in PBS + 3% ethanol) using the doses and schedules indicated.

Tumour Measurements

Animals were weighed twice weekly and xenograft tumour

measurements taken using digital calipers. Tumour volume was calculated using the formula (h x w x l)π /6. Measurements continued until the tumour reached the maximum allowable volume (i.e. $1cm^3$), Results were expressed as percentage tumour growth using day 1 tumour volumes as controls.

In the tests on the compounds shown in Table 6 and in Figures 9 to 17, the Methods used were as described in WO 94/29312. The following items a) to c) are also to be noted:

a) Standard ATase assay

ATase substrate DNA was prepared by incubation of purified calf thymus DNA with N-[^{3}H]-methyl-N-nitrosourea (18.7 Ci/mmole, Amersham International). Cell or tissue extracts were incubated with [^{3}H]-methylated-DNA substrate (100µl containing 6.7µg of DNA and 100fmol of O^6-[^{3}H]methylguanine) at 37°C for 60 mins.. Following acid hydrolysis of the DNA as previously described[33] the [^{3}H]-methylated protein was recovered and quantitated by liquid scintillation counting.

b) Drug Treatment

Mice were treated with the inactivator as a suspension in corn oil by intraperitoneal injection (i.p.) or by oral gavage (p.o.) 60 mins prior to temozolomide (100mg/kg in 20%DMSO in phosphate-buffered saline) which was always given by intraperitoneal injection: this schedule was repeated on days 1 to 5 inclusive. Controls received vehicle alone, inactivator alone or temozolomide alone.

c) Animals

The mice in the tests shown in Figure 11 and Table 8 were BALB-C derived athymic male mice (nu/nu athymic) from the in-house breeding colony of the Paterson Institute for Cancer Research as described in WO 94/29312 (Animal Services Unit-ASU Mice).

The mice in the tests shown in Figures 12-16 were Swiss mouse derived athymic male mice (o/nu athymic) as described above.

The mice in the tests shown in Figure 17 were DBA_2 mice from the in-house breeding colony of the Paterson Institute for Cancer Research (Animal Services Unit), originally from the Jackson Laboratory in 1970.

Test Results

The results of the ATase depletion assay on the compounds of Table 1 are shown in Table 2 or Table 3. Many of the compounds tested were more efficient in inactivating ATase than O^6-benzylguanine. In accordance with the results in WO 94/29312 the parent application, compounds in which R is a heterocyclic group were more efficient than the comparable compounds having benzyloxy side chains. In general the compounds in which RCH_2 is substituted or unsubstituted thenyl were the most efficient, the most preferred being halo- substituted thenyl having its halo substituent in a 1,3-relationship with the methyleneoxy group attached to the pyrimidine residue.

Tables 3, 4 and 5 summarize data for a number of parameters. Table 3 includes depletion assay results for recombinant ATase of the following types:

hAT = human
mAT = mouse
rAT = rat
chAT = Chinese hamster
ogt = *E. Coli* ogt gene
ada = *E. Coli* ada gene

The combinations of properties for the various inactivators can be seen in the tables. The following surprising points are noted in particular:

B.4316 is a compound of surprisingly high water solubility. B.4335 is a compound that is unexpectedly much more effective in the inactivation of ATase in Raji cells than of pure recombinant protein: generally, the I_{50} for inactivation of recombinant ATase *in vitro* is lower or similar to that in cultured cells.

B.4343 is a compound that has a very low I_{50} for ATase *in vitro* but is not as capable as agents with higher I_{50}s (e.g. B.4335) in the sensitization of Raji cells to the growth inhibitory effects of temozolomide. A similar example is B.4351 versus B.4349.

B.4316 was twice as effective as B.4280 but sensitization to temozolomide of Raji cells was almost identical. Thus different cell lines may respond surprisingly differently to these agents.

Figures 1 to 3 show that temozolomide, BCNU and fotemustine inhibit the growth of Raji cells in a dose-dependent manner but sensitivity is greatly increased by exposure to B.4316 at 0.1, 1.0 and 10μM respectively. In contrast B.4316 had no measurable effect on growth inhibition of Raji cells by melphalan or cisplatin (Fig. 4). This indicates that the inactivators were specifically sensitizing cells to the O^6-alkylating agents and not other classes of alkylating compound.

Figures 5 and 6 respectively show the B.4316 and B.4349 sensitization factors for the above therapeutic agents in Raji cells.

Figure 7 shows that of the inactivators examined human melanoma xenograft ATase depletion was complete only after administration of B.4314 and B.4351 under the experimental conditions used. The former was more effective in ATase depletion in liver and kidney of host animals whilst the latter was more effective in the brain, suggesting its relative ease in passing the blood-brain barrier. Noteworthy is the fact that whilst B.4311 was one of the most effective agents in sensitizing Raji cells to the

toxic effects of temozolomide, it was surprisingly one of the least effective agents in depleting mouse tissue or tumour xenograft ATase activity.

Figure 8 shows that B.4363 depletes ATase more effectively in human melanoma xenografts than in murine host tissues under the conditions used: relatively little effect was seen in brain tissue, suggesting its poor ability to cross the blood brain barrier.

The test results for the compounds of Table 6 (and some in Table 1) are shown in Table 7 and Figures 9 to 27.

B.4280, which is $\underline{O}^6$-(4-bromothenyl)guanine and has its bromo substituent in a 1, 3-relationship with the methylene group attached to the guanine residue, was more efficient in inactivating ATase *in vitro* than its 5-bromo analogue B.4269, in which the bromo substituent is in a 1, 4-relationship with the methylene group. Both B.4280 and B.4269 were more efficient than the unsubstituted thenyl derivative B.4205 despite having considerably larger $\underline{O}^6$ substituents.

Another preferred compound is B.4317 which is $\underline{O}^6$-(4-cyanothenyl)guanine. B.4317 is a more efficient inactivator *in vitro* than its 5-cyano analogue B.4283 or the unsubstituted thenyl derivative B.4205.

Typical ATase inactivation profiles for BeG and B.4205 and B.4280 are shown in Fig. 9.

The inactivation of ATase resulted in the sensitization of Raji cells to the growth inhibitory effects of temozolomide (Fig. 10). B.4280 was considerably more effective than either B.4205 or BeG in this respect.

ATase in human melanoma xenografts was inactivated by BeG, B.4205 and B.4280 (Fig. 11) with some indication that the rates of

recovery of ATase activity were different between the agents. B.4280 was the most effective *in vivo* inactivator at the doses examined.

B.4280 was able to inactivate ATase in most tissues as shown in Table 8. Thus, activity in brain, testis and bone marrow was near to control levels by 24 hours whereas lung and spleen activity had not completely recovered by 48 hours. Tumour activity was very low at 24 hours but had recovered completely by 48 hours. Differential recovery rates might be an important factor in the toxicity of ATase inactivators when used in combination with CNU or temozolomide.

Combinations of B.4205 or B.4280 and temozolomide given over three days were more effective in ATase inactivation in tumour xenografts than either agent alone (Fig. 12). Decreasing the dose of B.4205 had no major effect on the ability of the agent to inactivate ATase, 10mg/kg being as effective as 60mg/kg. B.4280 was more effective than B.4205 at equivalent doses. As before (Fig. 11) there was some indication that ATase recovery was less efficient in the tumour xenograft (Fig. 12) than in the liver (Fig. 13).

B.4205 (Fig. 14A) and B.4280 (Fig. 15A) were effective in sensitizing human melanoma xenografts to the growth inhibitory effects of temozolomide. A comparison of the two sets of data indicates that B.4280 was about twice as effective as B.4205 in this respect. At equi-effective doses for tumour growth inhibition, B.4280 seems to be less toxic than B.4205 (Figs. 14B and 15B).

In experiments using DBA_2 mice in combination with BCNU, B.4280 was considerably less acutely toxic than B.4205 or BeG as shown in Table 9. Oral administration of B.4280 was shown to be almost as effective as i.p. administration in sensitizing human melanoma xenografts to the growth inhibitory effects of temozolomide (Fig. 16A). Furthermore the oral combination appeared to be marginally less toxic than the i.p. route (Fig. 16B).

At a dose of 20mg/kg of inactivator in combination with temozolomide in DBA_2 mice, B.4205 and B.4280 were shown to be less acutely toxic than BeG, with B.4280 being less acutely toxic than B.4205 (Fig. 17).

Figures 18 and 19 show that B.4280 (PaTrin-2) (i.p. at 20 mg/kg and p.o at 30mg/kg respectively) depletes ATase in human melanoma xenografts more completely and for a more extensive period than it does in host tissues.

Figure 20 show that despite the considerably higher initial level of ATase activity in the human breast tumour, B.4280 depletes ATase therein more completely and for a longer period of time than in murine host tissues. In this study using 30mg/kg B.4280 i.p. extensive depletion was seen in brain tissue, indicating the ability to cross the blood-brain barrier.

Figure 21 likewise shows that despite the considerably higher initial level of ATase activity in the human prostate tumour, B.4280 depletes ATase therein more completely and for a longer period of time than in murine host tissues. In this study using 20mg/kg

B.4280 i.p. relatively little depletion was seen in brain tissue, indicating by reference to Figure 20 that the ability of B.4280 to cross the blood-brain barrier may be dose-dependent.

Figure 22 shows that B.4280 (20mg/kg i.p.) considerably increased the sensitivity of the human breast tumour xenograft to the growth inhibitory effects of temozolomide using a 5 day dosing schedule. This sensitization occurred despite the very high level of ATase in this tumour.

Figure 23 shows that a single dose of B.4280 (30mg/kg p.o.) considerably increased the sensitivity of the human melanoma tumour xenograft to the growth inhibitory effects of a single dose of the chloroethylating agent, fotemustine, without any substantial effect on toxicity.

Synthesis of O^6-(methylene[^{3}H])-(4-bromothenyl)guanine

Bromothenylaldehyde (0.79mg, 66.8 umoles was reacted with $NaB[^3H)_4$ (0.0167 mmoles, 60Ci/mmole) in isopropanol (350µl) for 1h at room temperature. The resulting [^{3}H]-4-bromothenylalcohol was extracted into pentane, dried, weighed and reacted with NaH (5.44mg), and the quaternary ammonium salt of guanine (15.55mg) in DMSO (250µl) for 1 hour at room temperature. The product was recovered by precipitation from acetic acid-ether (15µl glacial acetic in 1.5ml ether), washed with ether, dried and triturated with H_2O. After washing with water, the final product was dried to constant weight. Figure 28 shows the scheme for synthesis of the radio-labelled B.4280.

High performance liquid chromatography analysis

An aliquot of the product was dissolved in buffer A (10mM KH_2PO_4 containing 7.5% acetonitrile) and subjected to high performance liquid chromatography on an ODS-5 column. Elution at 1ml/min was with a linear gradient over 20 minutes from 100% A to 20% A:80% B (10mM KH_2PO_4 containing 80% acetonitrile). The effluent was monitored for UV absorption at 254nm and fractions (1

min) were collected and assayed for radioactivity after addiition of 10ml of Ecoscint A. It was shown that 96% of the radio activity co-chromatographed with authentic B.4280 (Figure 29).

Incubation of an aliquot of the product with known amounts of pure recombinant human ATase resulted in the transfer of radioactivity to the protein (Figure 30), strongly suggesting that the mechanism of ATase inactivation involves the transfer of the thenyl group to the active site cysteine residue in the ATase molecule. Measurement of the amount of radioactivity transferred to protein indicated that the $\underline{O}^6$-([^{3}H]-4-bromothenyl)guanine had a radiochemical purity of >96% and a specific activity of 16Ci/mmole.

$\underline{O}^6$([^{3}H]-4-bromothenyl)guanine can be used as an alternative to the standard method, which presently uses [^{3}H]-labelled substrate DNA, to determine the amounts of ATase, for example, in cell or tissue extracts. It may also be used to locate active ATase molecules in tumour and other tissue sections by incubation with such sections on microscope slides followed by washing, autoradiography and histological staining. It may also be used to monitor the formation of the [^{3}H]-labelled products of breakdown or metabolism of the agent after administration to mammals. It may also be used to determine the distribution of the B.4280 or its breakdown products in animal tissues and tumours by means of whole body autoradiography.

Typical synthetic procedures

Type 1A.

O^6-*(4-Bromothenyl)hypoxanthine*, B. 4292

4-Bromothenyl alcohol (1.16 g, 6 mmol) was added to sodium hydride (60% in oil ; 0.16 g, 2 mmol) and DMSO (1 ml). The solution was stirred for 30 min. The trimethylammonium salt (0.427 g , 2 mmol) was then added and stirring continued for 2.5 h at 20°C. The solution was cooled in an ice bath and poured into ether (60 ml) containing acetic acid (0.32 ml). A white precipitate was collected, triturated with water (4 ml) and collected again to give B. 4292 (436 mg , 69%) recrystallised from methanol.

Type 1B.

O^6-*Thenyl-2-methylhypoxanthine*, B. 4350

DABCO salt from 6-chloro-2-methylpurine:

6-chloro-2-methylpurine (0.5 g, 3 mmol) was dissolved in a mixture of DMF (5 ml) and diglyme (25 ml). DABCO (0.66 g, 6 mmol) was then added. The mixture was stirred for 1 h and the precipitate collected to give the quaternary salt (700 mg , 82%).
NMR (300 MHz, DMSO-d_6) : shift in ppm
2.65 (s), 3.27 (t, J=7.5 Hz), 3.78 (s), 4.14 (t, J=7.5 Hz), 8.21 (s).

Thenyl alcohol (684 mg , 6 mmol) was added to sodium hydride (60% in oil ; 80 mg , 2 mmol) and DMSO (0.5 ml). The solution was stirred for 30 min. The DABCO salt was then added and stirring continued for 5 h. The solution was then poured into ether (30 ml) containing acetic acid (0.15 ml). A precipitate was collected, triturated with water (4 ml) and collected again to give O^6-Thenyl-2-methylhypoxanthine (96 mg, 35%) recrystallised from acetonitrile.

Type 1C.

O^6-(4-Bromothenyl)-2-fluorohypoxanthine, B. 4353

To 3.6 ml of 40% fluoroboric acid precooled to -25°C in a bath was added O^6-(4-bromothenyl) guanine (326 mg, 1 mmol) with vigorous stirring. A solution of sodium nitrite (0.116 g, 1.7 mmol) in water (0.15 ml) was added dropwise over a period of 10 min. After 20 min, the solution was poured into ice. The mixture was then allowed to stand at 0°C for 15 h, then collected and dried to afford almost pure (t.l.c.) B. 4353 (180 mg, 55%). Flash chromatography (Hexane - Ethyl Acetate decreasing polarity little by little) afforded B. 4353 .

Typical synthetic procedures (continued)

Type 3D

O^4-*Thenyl-5-deazapterin*, B. 4376

a) N^2-*Pivaloyl-5-deazapterin*

A mixture of 5-deazapterin[33,34] (2.0g, 13.36mmol), 4-dimethylaminopyridine (0.22g, 1.8mmol) and pivalic anhydride (12ml) was heated to 165°C. Excess pivalic anhydride was distilled off and the residue dissolved in dichloromethane and applied to a pad of silica gel, and eluted with 2% methanol in dichloromethane. Evaporation and recrystallisation of the product from ethanol gave shiny cream coloured crystals (2.25g, 74%) of the pivaloyl derivative, m.p. 258-259°C ; λ_{max} (MeOH) 277 nm;
NMR (300MHz, DMSO-d_6) δ 1.28(s), 7.44(q), 8.43(dd), 8.88(dd), 11.4(s), 12.31(s).

b) N^2-*pivaloyl*-O^4-*thenyl-5-deazapterin* :

A suspension of N^2-pivaloyl-5-deazapterin (0.492g, 2mmol) in tetrahydrofuran (8ml) was stirred for 10 min, and tri-n-butylphosphine (0.606g, 3mmol), thenyl alcohol (0.432g, 3mmol) and diisopropylazodicarboxylate (0.606g, 3mmol) were added successively. The reaction was allowed to proceed for 2h at room temperature and evaporation then gave an oil. Hexane was added to induce crystallisation. Filtration and recrystallisation from hexane gave bright yellow crystals of the thenyl derivative (0.32g, 47%) m.p. 107-108°C ; λ_{max} (MeOH) 272, 311 nm;
NMR (300MHz, DMSO-d_6) δ 1.28(s), 5.86(s), 6.98(q), 7.28(dd), 7.43(dd), 7.52(q), 8.46(dd), 8.89(dd).

c) B. 4376

N^2-pivaloyl-O^4-thenyl-5-deazapterin (0.28g, 0.82mmol) was heated for 24h under reflux with aqueous NaOH (3M, 2ml) and ethanol (1ml). The solvent was removed by evaporation and the residual solid dissolved in water. Acidification with acetic acid gave a white precipitate. Filtration and recrystallisation of the solid from ethanol gave white crystals of O^4-thenyl-5-deazapterin (B. 4376), (0.107g, 51%).

Type 4D

O^6-*(4-Bromothenyl)-5-nitrocytosine*, B.4380

Sodium hydride (60% in oil; 80mg, 2mmol) was added to a stirred solution of 4-bromothenyl alcohol (290mg, 1.5mmol) in dry DMSO (1ml). After 30 min, 4-amino-2-chloro-5-nitropyrimidine[35] (174mg, 1mmol) was added and the mixture heated at 50°C for 2h. The DMSO was removed in vacuo and the pH adjusted to 7 with aqueous acetic acid. After extraction into ethyl acetate, the product B. 4380 was crystallised from methanol (51mg, 15%).

Type 5

S^6-*(4-bromothenyl)-6-thioguanine*, B.4352

Sodium hydride (60% in oil; 44mg, 1.1mmol) was added to a stirred solution of 4-bromothenyl mercaptan (418mg, 2mmol) in dry DMSO (0.5ml). After 30 min, 2-amino-*N,N,N*-trimethyl-1*H*-purin-6-aminium chloride (228mg, 1mmol) was added and stirring continued for 1h. Acetic acid (0.12ml) and ether (30ml) were added and after decantation and trituration with fresh ether, B.4352 (38mg, 11%) was filtered off.

9-Substituted O^6-(4-bromothenyl)guanines:

O^6-*(4-Bromothenyl)-9-(ethoxymethyl)guanine*, B.4369

O^6-(4-Bromothenyl)guanine (652mg, 2mmol) was dissolved in sodium ethoxide (1M; 2ml, 2mmol). After 10 min, the ethanol was removed and the residue was dissolved in dry DMF. Chloromethyl ethyl ether (189mg, 2mmol) was added dropwise to the stirred solution under an atmosphere of argon. After 45 min, the solvent was removed. The oily product was crystallised from ethanol giving B.4369 (158mg) as needles. A further 118mg was obtained by flash chromatography of the mother liquor on silica gel with 5% ethanol in CH_2Cl_2. Total yield, 39%.

O^6-*(4-Bromothenyl)-9-(2-hydroxyethoxymethyl)guanine*, B. 4335

A stirred mixture of *O^6*-(4-bromothenyl)guanine (294mg, 1mmol), $(NH_4)_2SO_4$ (47mg) and hexamethyldisilazane (5ml) was heated at reflux for 3h. Volatile material was then evaporated under vacuum. The residue was stirred with benzene (15ml) and $Hg(CN)_2$ (344mg, 1.3mmol) under reflux for 30 min. A solution of (2-acetoxyethoxy)methyl bromide {Ref 4 p33} (197mg, 1mmol) in benzene (10ml) was added, reflux maintained for 2h, and the cloudy diluted with chloroform (150ml). The organic phase was washed with saturated aqueous $NaHCO_3$ (30ml), followed by KI (1M ; 30ml), dried over $MgSO_4$ and evaporated to give an oil (313mg). This oil was chromatographed on a silica gel column with $CHCl_3$-MeOH (12 : 1) as eluant, yielding almost pure (t.l.c.) O-acetate (141mg) of B. 4335.
Methanol (60ml) was saturated with dry ammonia and poured onto this O-acetate in a flask which was tightly stoppered. After dissolution, stirring was stopped and the flask left closed overnight. Evaporation of methanol gave B. 4335 (135mg , 46%), recrystallised from isopropanol.

O^6-*4-bromothenyl-9-(β-D-ribofuranosyl)guanine*, B.4363

A mixture of 2',3',5'-tri-(O-acetyl)guanosine[36] (409mg, 1mmol), tri-n-butylphosphine (303mg, 1.5mmol) and 4-bromothenyl alcohol (290mg, 1.5mmol) in dry tetrahydrofuran (16ml) was stirred at room temperature for 45 min. Then diisopropyl azodicarboxylate (303mg, 1.5mmol) was added dropwise and the mixture stirred for 2h. The solution was evaporated leaving an oil which was dissolved in THF/MeOH/25% aqueous ammonia (1:1:1 ; 5 ml) and kept for 48 h at 4°C.

Adsorption on silica gel and column chromatography with $CHCl_3$/MeOH (15:1 to 10:1) gave the riboside B.4363 (205mg, 44%).

O^6-4-Bromothenyl-9-(β-D-2'-deoxyribofuranosyl)guanine, B.4379.

A mixture of 3',5'-di-(O-acetyl)-2'-deoxyguanosine[37] (554mg, 1.5mmol), tri-n-butylphosphine (666.6mg, 3.3mmol) and 4-bromothenyl alcohol (638mg, 3.3mmol) in dry tetrahydrofuran (40ml) was stirred at 80°C for 15 min. Then diisopropyl azodicarboxylate (666.6mg, 3.3mmol) was added dropwise and 15 min later, the reaction mixture was cooled and evaporated leaving an oil. This was dissolved in THF/MeOH/25% aqueous ammonia (1:1:1 ; 5 ml) and kept for 48 h at 4°C. Adsorption on silica gel and column chromatography with $CHCl_3$/MeOH (20:1) gave the 2'-deoxyriboside B.4379 (338mg, 51%).

9-(β-D-Arabinofuranosyl)-O^6-(4-bromothenyl)guanine, B.4368.

An alkoxide solution was made from sodium hydride (60% in oil; 60mg, 1.5mmol) and 4-bromothenyl alcohol (344mg, 1.8mmol) in dry DMSO (0.5ml) over 1 h. It was reacted with 2-amino-9-(β-*D*-arabinofuranosyl)-6-chloropurine[38] (151mg, 0.5mmol) and stirred for 5 min at room temperature, then 15 min at 60-65°C. Cooling and trituration with ether (50ml) and filtration yielded a solid which was dissolved in water (5ml), neutralised with acetic acid and treated with silica gel. Column chromatography with ethyl acetate/MeOH (19:1) gave the arabinoside B.4368 (87mg, 38%), pure on t.l.c..

O^6-substituted guanines

These were made by the standard procedure from the quaternary salt 2-amino-*N*,*N*,*N*-trimethyl-1*H*-purin-6-aminium chloride and the appropriate alkoxide derived from the alcohol and sodium hydride in DMSO (cf.pp.16d, 17, 18, 47 of 7/12/95).

TABLE 1A

Compound, Test No.	O^6-Substituent RCH_2	Yield %	Solvent for Recrystn.	M.p. (decomp.) (°C)	Formula	Molecular Weight		Analysis C	H	N
Type 1A. Hypoxanthines										
B. 4293	furfuryl	60	MeOH	154	$C_{10}H_8N_4O_2$	216				
B. 4291	thenyl	66	MeOH	168	$C_{10}H_8N_4OS$	232	Found	51.85	3.40	24.12
							Req.	51.71	3.47	24.12
B. 4292	4-bromothenyl	69	MeOH	170	$C_{10}H_7BrN_4OS$	311	Found	38.33	2.18	17.66
							Req	38.6	2.26	18.00
1B. *2-Methylhypoxanthines*										
B. 4347	benzyl	43	MeCN	191-193[a]	$C_{13}H_{12}N_4O$	240	Found	65.05	4.91	23.30
							Req	64.99	5.03	23.32
B. 4350	thenyl	35	MeCN	176-178[a]	$C_{11}H_{10}N_4OS$	325	Found	53.63	3.90	22.67
							Req	53.64	4.09	22.75
1C. *2-Fluorohypoxanthines*										
B.4353	4-bromothenyl	55	Column	142	$C_{10}H_6BrFN_4OS$	329				
1D. *9-(2-Hydroxyethoxy-methyl)guanines*										
B. 4334	benzyl	46	i-PrOH	150-152[a]	$C_{15}H_{17}N_5O_3$	315	Found	57.19	5.59	21.93
							Req	57.13	5.43	22.21
B. 4335	4-bromothenyl	42	i-PrOH	156-158[a]	$C_{13}H_{14}BrN_5O_3S$	400	Found	39.16	3.68	17.20
							Req	39.01	3.53	17.50
1E. *8-Hydroxyguanines*										
B.4349	4-bromothenyl	56	Aq. EtOH	> 230	$C_{10}H_8BrN_5O_2S.½\ H_2O$	351	Found	34.53	2.48	19.50
							Req.	34.20	2.58	19.94
Type 2A. *8-Azaguanines*										
B.4270	4-fluorobenzyl	40	Aq. MeOH	> 280	$C_{11}H_9FN_6O$	260	Found	51.50	3.85	29.44
							Req.	50.77	3.49	32.30
B.4314	4-chlorothenyl	26	Aq. MeOH	> 200	$C_9H_7ClN_6OS$	282.7	Found	38.86	2.61	28.61
							Req.	38.24	2.50	29.73
B.4289	4-bromothenyl	12	MeCN	> 190	$C_9H_7BrN_6OS$	327	Found	35.91	2.78	24.60

TABLE 1A (continued)

Compound, Test No.	O^6-Substituent RCH_2	Yield %	Solvent for Recrystn.	M.p. (decomp.) (°C)	Formula	Molecular Weight		Analysis C	H	N
							Req.	33.04	2.16	25.69
2B. *8-Aza-7-deazaguanines*										
B. 4310	benzyl		MeOH	160	$C_{12}H_{11}N_5O.H_2O$	259	Found	55.53	4.9	26.41
							Req.	55.59	5.01	27.02
B. 4340	4-fluorobenzyl	65	EtOH	188	$C_{12}H_{10}FN_5O.¼H_2O$	263.7	Found	53.82	3.76	26.97
							Req.	54.6	4.0	26.55
B. 4339	4-chlorobenzyl	92	EtOH	242-244[a]	$C_{12}H_{10}ClN_5O.$ $½H_2O.¼EtOH$	296	Found	51.15	3.89	23.43
							Req.	50.7	4.25	23.64
B. 4343	piperonyl	50	EtOH	186						
B. 4348	furfuryl		EtOH	150[a]	$C_{13}H_{11}N_5O_3$	285	Found	54.52	3.82	24.50
							Req.	54.7	3.88	24.55
B. 4338	thenyl	68	EtOH	180	$C_{10}H_9N_5O_2.¼H_2O$	235.7	Found	50.96	3.87	29.54
							Req.	50.96	4.06	29.71
B. 4337	4-bromothenyl	79	EtOH	180	$C_{10}H_9N_5OS$	247	Found	47.58	3.54	27.41
							Req.	47.7	3.8	27.8
					$C_{10}H_8BrN_5OS$	326	Found	37.08	2.51	21.31
							Req.	36. 8	2.5	21.5
Type 3A. *8-Oxaguanines*										
B. 4272	4-fluorobenzyl	41	Acetone	223-224	$C_{11}H_8FN_5O_2$	261	Found	50.39	3.08	26.65
							Req.	50.58	3.09	26.81
B. 4285	4-chlorobenzyl	63	Acetone	219-220	$C_{11}H_8ClN_5O_2$	277.7	Found	47.59	2.88	25.25
							Req.	47.58	2.90	25.22
B. 4299	4-chlorothenyl	55	Acetone	164-165	$C_9H_6ClN_5O_2S$	283.7	Found	37.68	2.15	24.43
							Req.	38.10	2.13	24.69
B. 4287	4-bromothenyl	61	Acetone	170-172	$C_9H_6BrN_5O_2S$	328	Found	33.30	1.85	21.37
							Req.	32.94	1.84	21.34
3B. *8-Thiaguanines*										
B. 4296	benzyl	39	EtOH		$C_{11}H_9N_5OS$	259				
B.4286	4-fluorobenzyl	11	PLC	182-184	$C_{11}H_8FN_5OS$	277				

TABLE 1A (continued)

Compound, Test No.	O^6-Substituent RCH_2	Yield %	Solvent for Recrystn.	M.p. (decomp.) (°C)	Formula	Molecular Weight		Analysis C	H	N
B. 4315	4-chlorothenyl	13	MeOH		$C_9H_6ClN_5OS_2$	299.8	Found	36.27	2.04	23.07
							Req.	36.06	2.02	23.36
B. 4351	4-bromothenyl	41	MeOH	156-160	$C_9H_6BrN_5OS_2$	344	Found	31.49	1.60	20.11
							Req.	31.41	1.76	20.35
3C. *Pterins (O^4-substituent)*										
B. 4290	4-fluorobenzyl	55	MeOH	>210	$C_{13}H_{10}FN_5O$	271	Found	57.87	3.88	25.65
							Req.	57.56	3.72	25.82
B. 4316	4-chlorothenyl	41	MeOH	>170	$C_{11}H_8ClN_5OS$	293.7	Found	44.93	2.84	23.72
							Req.	44.98	2.75	23.84
B. 4288`	4-bromothenyl	63	MeOH	178-179	$C_{11}H_8BrN_5OS$	338	Found	39.34	3.13	20.25
							Req.	39.07	2.38	20.71
Type 4A. *2,4-diamino-6-hydroxypyrimidines*										
B. 4305	4-fluorobenzyl	98	C_6H_6/Petrol	133-134	$C_{11}H_{11}FN_4O$	234	Found	56.20	4.79	23.66
							Req.	56.40	4.73	23.92
B. 4304	4-chlorobenzyl	31	C_6H_6	122-123	$C_{11}H_{11}ClN_4O$	250.7	Found	52.43	4.56	22.47
							Req.	52.70	4.42	22.35
B. 4303	piperonyl	79	MeCN	168-171	$C_{12}H_{12}N_4O_3$	260	Found	55.31	4.64	21.38
							Req.	55.38	4.65	21.52
B. 4307	thenyl	97	MePh	100	$C_9H_{10}N_4OS$	222	Found	48.83	4.58	25.25
							Req.	48.63	4.54	25.21
B. 4302	4-chlorothenyl	45	MePh	129-130	$C_9H_9ClN_4OS$	256.7	Found	42.40	3.68	22.00
							Req.	42.11	3.53	21.83
4B. *2,4-Diamino-6-hydroxy-5-nitrosopyrimidines*										
B.4301	4-fluorobenzyl	76	MeOH	>250	$C_{11}H_{10}FN_5O_2$	263	Found	49.60	3.90	26.29
							Req.	50.19	3.83	26.61
B. 4311	4-chlorothenyl	84	Acetone	>190	$C_9H_8ClN_5O_2S$	285.7	Found	37.54	2.79	24.22

TABLE 1A (continued)

Compound, Test No.	O^6-Substituent RCH_2	Yield %	Solvent for Recrystn.	M.p. (decomp.) (°C)	Formula	Molecular Weight		Analysis C	H	N
B. 4312	4-bromothenyl	62	Acetone	200-201	$C_9H_8BrN_5O_2S$	330	Req.	37.84	2.82	24.51
							Found	32.87	2.38	20.96
							Req.	32.74	2.44	21.21
4C. *2,4-Diamino-6-hydroxy-5-nitropyrimidines*										
B. 4308	piperonyl	67	DMF	>175	$C_{12}H_{11}N_5O_5$	305	Found	47.44	4.07	22.83
							Req.	47.22	3.63	22.94
B. 4306	thenyl	34	MeOH	159-160	$C_9H_9N_5O_3S$	267	Found	40.99	3.71	25.99
							Req.	40.44	3.39	26.21

Continuation of Table 1a

Compound, Test No.	O^6-Substituent RCH_2	Yield %	Solvent for Recrystn.	M.p. (decomp.) (°C)	Formula	Molecular Weight		Analysis C	H	N
Type 3D. 5-Deazapterins (O^4-substituent)										
B. 4376	thenyl	51	EtOH	215-216	$C_{12}N_{10}N_4OS$	258				
4D. *5-Nitrocytosines (O^2-substituent)*										
B. 4380	4-bromothenyl	15	MeOH	143-144	$C_9H_7BrN_4O_3S$	331				
5. *6-Thioguanines (S^6-substituent)*										
B. 4228	piperonyl	69	CH_3OH	204-212	$C_{13}H_{11}N_5O_2S$	301	Found	50.25	3.60	23.66
							Req.	51.82	3.68	23.24
B. 4352	4-bromothenyl	11	CH_3OH	180-184	$C_{10}H_8BrN_5O_3 \cdot \frac{1}{3} CH_3OH$	342	Found	35.07	2.42	19.49
							Req.	35.16	2.66	19.84

TABLE 1A (continued)

Compound, Test No.	9-Substituent	Yield %	Solvent for Recrystn.	M.p. (°C)	Formula	Molecular Weight	Analysis[a] C	H	N
B.4369	ethoxymethyl	39	EtOH	134-5	$C_{13}H_{14}BrN_5O_2S$	384	40.58 (40.64	3.71 3.67	17.97 18.23)
B.4370	*n*-octyloxymethyl	39	EtOH	90	$C_{19}H_{26}BrN_5O_2S$	468	48.97 (48.72	5.67 5.60	14.82 14.95)
B.4334[b]	2-hydroxy-ethoxymethyl	46	*i*-PrOH	150-2	$C_{15}H_{17}N_5O_3$	315	57.19 (57.13	5.59 5.43	21.93 22.21)
B.4335	2-hydroxy-ethoxymethyl	42	*i*-PrOH	156-8	$C_{13}H_{14}BrN_5O_3S$	400	39.16 (39.01	3.68 3.53	17.20 17.50)
B.4363	β–D-ribo-furanosyl	44			$C_{15}H_{16}BrN_5O_5S$	458			
B.4368	β–D-arabino-furanosyl	38			$C_{15}H_{16}BrN_5O_5S$	458			
B4379	β–D-2-deoxyribo-furanosyl	51			$C_{15}H_{16}BrN_5O_4S$	442			

[a] Found, with required values in parenthesis.
[b] O^6-benzyl

TABLE 1B

Compound Type, Test No.	O^6-Substituent RCH_2	λ_{max} (MeOH) (nm)	δ_H[ppm from TMS, $(CD_3)_2SO$,] J(Hz)
Type 1A. *Hypoxanthines*			
B. 4293	furfuryl	252	5.60(s), 6.53(dd, 3.1, 1.9), 6.69(d, 3.1), 7.76(dd, 1.9, 0.9) 8.39(s), 8.55(s)
B. 4291	thenyl	240	5.83(s), 7.08(dd, 5.1, 3.4), 7.35(d, 3.4), 7.6(d, 5.1), 8.39(s), 8.51(s)
B. 4292	4-bromothenyl	251	5.80(s), 7.38(d,1.3), 7 73(d, 1.3), 8.42(s), 8.58(s)
Type 1B. *2-Methylhypoxanthines*			
B. 4347	benzyl	256	2.61(s), 5.60(s), 7.50(m), 8.32(s)
B. 4350	thenyl	240	2.63(s), 5.77(s), 7.05(dd, 5.1, 2.4, 7.33(d), 2.4), 7.58(dd, 5.1, 1.0) 8.26(s), 13.22(s)
Type 1C. *2-Fluorohypoxanthines*			
B. 4353	4-bromothenyl	233, 255	5.77(s), 7.4(d, 1.5), 7.77(d, 1.5), 8.45(s), 13.64(bs).
Type 1D. *9-(2-Hydroxyethoxymethyl)guanines*			
B. 4334	benzyl	247, 283	3.48(m), 4.70(s), 5.45(s), 6.59(s), 7.45(m), 8.03(s),
B. 4335	4-bromothenyl	245, 284	3.49(m), 4.71(s), 5.45(s), 5.66(s), 6.65(s), 7.30(d, 1.5) 7.72(d, 1.5) 8.04(s)
Type 1E. *8-Hydroxyguanines*			
B. 4349	4-bromothenyl	239, 293	5.54(s), 6.24(s), 7.33(d, 1.4) 7.70(d, 1.4), 10.49(s) 11.12(s)
Type 2A. *8-Azaguanines*			
B. 4270	4-fluorobenzyl	288	5.57(s), 7.04(s), 7.28(m), 7.65(m), 15.38(s).
B. 4314	4-chlorothenyl	288	5.71(s), 7.13(s), 7.41(d, 1.5), 7.66(d, 1.5), 15.42(s).
B. 4289	4-bromothenyl	287	5.73(s), 7.12(s), 7.43(d, 1.5), 7.76(d, 1.5), 15.39(s).

TABLE 1B (continued)

Type 2B. *8-Aza-7-deazaguanines*			
B. 4310	benzyl	277	5.50(s), 6.68(s), 7.74(m), 7.82(s), 12.87(bs)
B. 4340	4-fluorobenzyl	278	5.49(s), 6.70(s), 7.20(m), 7.61(m), 7.82(s), 12.88(bs)
B. 4339	4-chlorobenzyl	276	5.50(s), 6.69(s), 7.49(d, 8.4), 7.56(d, 8.4), 7.83(s) 12.90(s)
B. 4343	piperonyl	282	5.39(s), 6.05(s), 6.69(s), 6.94(d, 7.9) 7.04(dd, 7.9, 1.5), 7.14(d, 1.5), 7.80(s) 12.86(bs).
B. 4348	furfuryl	277	5.46(s), 6.52(s), 6.70(s), 6.71(s), 7.73(s), 7.79(s), 12.85(bs).
B. 4338	thenyl	278	5.69(s), 6.73(s), 7.07(d, 3.5), 7.35(s), 7.60(d, 1.1), 7.79(s), 12.90(bs).
B. 4337	4-bromothenyl	278	5.65(s), 6.76(s), 7.38(s), 7.72(d, 1.3), 7.81(s), 12.91(bs).
Type 3A. *8-Oxaguanines*			
B. 4272	4-fluorobenzyl	257, 341	5.62(s), 7.30(t, 9.1), 7.68(m), 7.91(s), 7.97(s).
B. 4285	4-chlorobenzyl	256, 340	5.63(s), 7.53(d, 8.3), 7.65(d, 8.3), 7.90(s), 7.97(s).
B. 4299	4-chlorothenyl	252, 343	5.78(s), 7.46(d, 1.6), 7.72(d, 1.6), 7.95(s), 8.01(s).
B. 4287	4-bromothenyl	253, 343	5.79(s), 7.49(d, 1.6), 7.81(d, 1.6), 7.95(s), 8.01(s)
Type 3B. *8-Thiaguanines*			
B. 4296	benzyl	227, 361	
B. 4286	4-fluorobenzyl	235, 362	5.59(s), 7.29(t 8.9), 7.51(bs), 7.67(m)
B. 4315	4-chlorothenyl	228, 360	5.75(s), 7.44(d, 1.6), 7.55(bs), 7.69(d, 1.6)
B. 4351	4-bromothenyl	228, 361	5.78(s), 7.45(d, 1.6), 7.46(bs), 7.75(d, 1.6)
Type 3C. *Pterins (C^4-substituent)*			
B. 4290	4-fluorobenzyl	232, 264(sh), 362	5.56(s), 7.29(t, 8.85), 7.44(bs), 7.66(m), 8.45(d, 1.8), 8.82(d, 1.8).
B. 4316	4-chlorothenyl	232, 364	5.71(s), 7.41(d, 1.6), 7.47(bs), 7.67(d, 1.6), 8.46(d, 2.0), 8.83(d,2.0)
B. 4288	4-bromothenyl	231, 364	5.73(s), 7.44(d, 1.6), 7.50(bs), 7.77(d, 1.6) 8.46(d, 2) 8.83(d, 2).

TABLE 1B (continued)

Type 4A. *2,4-diamino-6-hydroxy-pyrimidines*			
B. 4305	4-fluorobenzyl	238, 267	5.10(s), 5.19(s), 5.96(s), 6.10(s), 7.19(t, 8.8), 7.44(dd, 8.8, 5.8).
B. 4304	4-chlorobenzyl	238, 268	5.11(s), 5.22(s), 5.96(s), 6.10(s), 7.44(s).
B. 4303	piperonyl	236, 267	5.09(s), 5.11(s), 5.97(s), 6.03(s), 6.07(s), 6.91(d, 1.1), 7.00(s).
B. 4307	thenyl	235, 267	5.08(s), 5.40(s), 6.00(s), 6.10(s), 7.03(dd, 8.1, 3.5)7.20(dd, 8.1, 1.1), 7.54 (dd, 3.5, 1.1).
B. 4302	4-chlorothenyl	236, 265	5.08(s), 5.35(s), 6.03(s), 6 13(s), 7.19(s), 7.55(d 1.6).
Type 4B. *2,4-Diamino-6-hydroxy-5-nitrosopyrimidines*			
B. 4301	4-fluorobenzyl	336	5.59(s), 7.26(m), 7.65(m), 7.80(bs), 7.85(bs), 8.00(bs), 10.05(bs).
B. 4311	4-chlorothenyl	335	5.73(s), 7.40(d, 1.6), 7.66(d, 1.6), 7.94(s), 7.98(d, 2.7), 8.11(d, 4.2) 10.03 (d, 4.2).
B. 4312	4-bromothenyl	335	5.75(s), 7.42(d, 1.4), 7.75(d, 1.4), 7.93(s), 7.98(s), 8.12(d, 4.0), 10.04 (d, 4.0)
Type 4C. *2,4-diamino-6-hydroxy-5-nitropyrimidines*			
B. 4308	piperonyl	288,330	5.33(s), 6.05(s), 6.95(d, 8.0), 7.00(dd, 8.0, 1.4), 7.10(d, 1.4); 7.26(bs), 7.3 7.96(bs).
B. 4306	thenyl	234, 329	5.59(s), 7.03(dd, 5.1, 3.5), 7.28(d, 3.5), 7.32(bs), 7.56(d, 5.1), 7.94(bs).

TABLE 1B (continued)

Compound Type, Test No.	Substituent RCH_2	λ_{max} (MeOH) (nm)	δ_H[ppm from TMS, $(CD_3)_2SO$,]J(Hz)
Type 3D *5-Deazapterins (O^4-substituent)*			
B.4376	thenyl	248, 309	5.54(s), 6.96(q), 7.716(dd), 7.38(dd), 7.41(q), 8.39(dd), 8.79(dd).
Type 4D *5-Nitrocytosines (O^2-substituent)*			
B.4380	4-bromothenyl	255 sh, 334	5.19(s), 7.20(s), 7.56(d), 8.24(s), 8.70(s), 8.90(s).
Type 5 *6-Thioguanines (S^6-substituent)*			
B.4228	piperonyl	245, 311	4.56(s), 6.06(s), 6.55(s), 7.03(d), 7.06(d), 7.14(s), 8.08(s), 12.67(bs).
B.4352	4-bromothenyl	241, 314	4.77(s), 6.52(s), 7.18(d), 7.51(d), 7.93(s), 12.61(bs

TABLE 1B (continued)

Compound Type, Test No.	9-Substituent Yield %	λ_{max} (MeOH) (nm)	δ_H[ppm from TMS, $(CD_3)_2SO$,]J(Hz)
B.4369	ethoxymethyl	245, 284	3.35(s), 5.41(s), 5.66(s), 6.66(s), 7.38(d), 7.73(d), 8.04(s).
B.4370	*n*-octyloxymethyl	245, 284	0.09(t), 1.17(m), 3.36(t), 5.41(s), 5.66(s), 6.66(s), 7.38(d), 7.72(d), 8.03(s).
B.4334 [a]	2-hydroxy-ethoxymethyl	245, 283	3.48(m), 4.70(s), 5.45(s), 6.59(s), 7.45(s), 8.03(s).
B.4335	2-hydroxy-ethoxymethyl	245, 284	3.49(m), 4.71(s), 5.45(s), 5.66(s), 6.65(s), 7.30(d. 1.5), 7.72(d, 1.5), 8.40(s).
B.4363	β–D-ribo-furanosyl	-	3.54(m), 3.63(m), 3.91(dd), 4.12(dd), 4.48 (ddd), 5.12(dd), 5.18(d), 5.45(d), 5.66(s), 5.80(dd), 6.61(s), 7.38(d), 7.71(d), 8.15(s).
B.4368	β–D-arabino-furanosyl	245, 284	3.64(m), 3.76(dd), 4.07(m), 5.09(dd), 5.51(d), 5.53(m), 6.13(d), 6.60(d), 7.37(d), 7.71(d), 7.95(s)
B.4379	β–D-2-deoxyribo-furanosyl		2.39(ddd), 2.72(ddd), 3.65(ddd), 3.98(dd), 4.40(dd), 5.11(s) 5.41(d), 5.80(s), 6.38(dd), 6.67(s), 7.49(d), 7.83(d), 8.25(s).

[a] O^6-benzyl

TABLE 2

INACTIVATOR TYPE	I_{50}(μM) hAT	T 1/2 (h) in PBS
1A		
B.4291 O^6-(thenyl)-hypoxanthine	1.9	>20
B.4293 O^6-(furfuryl)-hypoxanthine	28	>16
B.4292 O^6-(4-bromothenyl)-hypoxanthine	0.3	>16
O^6-(benzyl)-hypoxanthine [b]	85	
1B		
B.4347 O^6-(benzyl)-2-methylhypoxanthine	75	
B.4350 O^6-(thenyl)-2-methylhypoxanthine	14	
1C		
B.4353 O^6-(4-bromothenyl)-2-fluorohypoxanthine	1.4	
O^6-(benzyl)-2-fluorohypoxanthine [a]	48	
1D		
B.4334 O^6-(benzyl)-9-(2-hydroxyethoxymethyl) guanine	8	>20
B.4335 O^6-(4-bromothenyl)-9-(2-hydroxy ethoxymethyl)guanine	See Table 3	
1E		
B.4349 O^6-(4-bromothenyl)-8-hydroxyguanine	See Table 3	
O^6-(benzyl)-8-hydroxyguanine [a]	0.3	
2A		
B.4270 O^6-(4-fluorobenzyl)-8-azaguanine	0.08	

TABLE 2 (continued)

INACTIVATOR TYPE	I_{50}(μM) hAT	T 1/2 (h) in PBS
B.4314 O^6-(4-chlorothenyl)-8-azaguanine	See Table 3	
B.4289 O^6-(4-bromothenyl)-8-azaguanine	0.045	>10
O^6-(benzyl)-8-azaguanine [a]	0.07	
2B		
B.4310 O^6-(benzyl)-7-deaza-8-azaguanine	0.01	>16
B.4340 O^6-(4-fluorobenzyl)-8-aza-7-deazaguanine	0.018	>16
B.4339 O^6-(4-chlorobenzyl)-8-aza-7-deazaguanine	0.02	1.5
B.4343 O^6-(piperonyl)-8-aza-7-deazaguanine	See Table 3	
B.4348 O^6-(furfuryl)-8-aza-7-deazaguanine	0.036	0.27
B.4338 O^6-(thenyl)-8-aza-7-deazaguanine	0.01	
B.4337 O^6-(4-bromothenyl)-8-aza-7-deazaguanine	0.007	>20
3A		
B.4272 O^6-(4-fluorobenzyl)-8-oxaguanine	See Table 3	
B.4285 O^6-(4-chlorobenzyl)-8-oxaguanine	0.225	4.6
B.4299 O^6(4-chlorothenyl)-8-oxaguanine	0.243	9.2
B.4287 O^6-(-4-bromothenyl)-8-oxaguanine	0.24	2.6
B.4232 O^6-(benzyl)-8-oxaguanine	0.25	

TABLE 2 (continued)

INACTIVATOR TYPE	I_{50}(μM) hAT	T 1/2 (h) in PBS
3B		
B.4296 O^6-(benzyl)-8-thiaguanine	0.02	>17
B.4286 O^6-(4-fluorobenzyl)-8-thiaguanine	0.03	>17
B.4315 O^6-(4-chlorothenyl)-8-thiaguanine [c]	0.006	
B.4351 O^6-(4-bromothenyl)-8-thiaguanine	See Table 3	
3C		
B.4290 O^4-(4-fluorobenzyl)-pterin	0.088	>10
B.4316 O^4-(4-chlorothenyl)-pterin	See Table 3	
B.4288 O^4-(4-bromothenyl)-pterin	0.025	>10
4A		
B.4305 2,4-diamino-6-(4-fluorobenzyloxy)pyrimidine	4.0	>16
B.4304 2,4-diamino-6-(4-chlorobenzyloxy)pyrimidine	5.0	>16
B.4303 2,4-diamino-6-(3,4-piperonyloxy)pyrimidine	0.8	12.5
B.4307 2,4-diamino-6-(thenyloxy)pyrimidine	0.4	4.2
B.4302 2,4-diamino-6-(4-chlorothenyloxy)pyrimidine	0.17	>16
2,4-diamino-6-(benzyloxy)pyrimidine [a]	15	
4B		
B.4301 2,4-diamino-6-(4-fluorobenzyloxy)-5-nitrosopyrimidine	0.0175	>16

TABLE 2 (continued)

INACTIVATOR TYPE	I_{50}(uM) hAT	T 1/2 (h) in PBS
B.4311 2,4-diamino-(4-chlorothenyloxy)-5-nitrosopyrimidine	See Table 3	
B.4312 2,4-diamino-6-(4-bromothenyloxy)-5-nitrosopyrimidine	0.045	4
2,4-diamino-6-(benzyloxy)-5-nitrosopyrimidine [a]	0.06	
4C		
B.4306 2,4-diamino-6-(thenyloxy)-5-nitropyrimidine	2.3	>16
B.4308 2,4-diamino-6-piperonyloxy-5-nitropyrimidine	0.5	9.2
2,4-diamino-6-benzyloxy-5-nitropyrimidine [a]	0.06	
4D		
B.4380 O^2(4-bromothenyl)-5-nitrocytosine	50	
5		
B.4228 S^6-(piperonyl)-6-thioguanine	50	
B.4352 S^6-(4-bromothenyl)-6-thioguanine	8	
Comparative		
B.4376 O^6-thenyl-5-deazapterin	1,600	
Results for some 9-substituted O^6 (4-bromothenyl)guanines are included in Table 7.		

a Data taken from Chae et al, J. Med. Chem. 1995, 38, 359-365
b Data taken from Moschel et al., J. Med. Chem. 1992, 35, 4486-4491.
c B.4315 Raji I_{50} (uM) 0.002

Blank Space = not done.

TABLE 3

Inactivator	Mol Wgt	I_{50} hAT (μM)	I_{50} Raji (μM)	I_{50} mAT (μM)	I_{50} rAT (μM)	I_{50} chAT (μM)	I_{50} ogt (μM)	I_{50} ada (μM)	$T^{1/2}$ (h) PBS	$T^{1/2}$(h) by Assay	Raji cell sensitisation factor (D_{50} control / D_{50} 'B')					Solubility in Water (mg/ml)	Raji cell toxicity at 10 μM 'B' alone (% Growth)
											BCNU	TEMOZOLOMIDE					
											Inactivator concentration (μM)						
											10	10	1.0	0.5	0.1		
B4272	261	0.05	0.023	0.125	0.075	0.04	>1000	>1000	5.7	2.6	1.88	1.41	---	---	---	0.002	111.21+23.3
B4311	286	0.009	0.009	0.008	0.016	0.02	1.8	>1000	10	12.5	8.0	73.3	8.25	---	1.4	0	113.0+31.0
B4314	283	0.011	0.012	0.073	0.037	0.03	2	>1000	>19	>48	7.62	84	4.61	---	3.46	Not done	55.5+7.3 (D_{50} 16 μM)
B4316	294	0.025	0.011	0.068	0.03	0.04	3.8	>1000	>19	32	6.4	66	13.2	---	1.4	0.3	85.5+20.0
B4335	400	0.33	0.07	15.63	6.5	1.8	156	>1000	>19	>48	5.33	38	3.5	---	1.0	0.009	98.4+12.2
B4343	285	0.007	0.0085	0.31	0.045	0.02	30	>1000	7.5	3	3.81	9.5	2.12	---	1.6	0.01	97.0+10.0
B4349	342	0.018	0.007	0.043	0.074	0.02	0.08	>1000	7.3	>48	4.8	50.8	33	---	2.4	0.002	90.0+13.0
B4351	344	0.003	0.005	0.071	0.027	0.03	5.8	>1000	>16	12	4.8	18.1	1.32	---	1.2	Not done	117.5+29.1
BeG	241	0.04	0.1	0.2	0.076	0.01	17	>1000	>64	>75	4.33	27.5	1.89	---	1.03	0.023	82.2+11.0
PaTrin-2 B.4280	326	0.003	0.003	0.05	0.019	0.03	0.85	>1000	>16	>48	6.0	60	33	8	5.5	Not done	69.8+10.3 (D50 44μM)

--- = Not Done

TABLE 4

EFFECT OF INACTIVATOR PRETREATMENT ON SENSITISATION OF VAROUS HUMAN CANCER CELL LINES TO TEMOZOLOMIDE

INACTIVATOR	SENSITISATION FACTOR (D_{50} control/D_{50} 'B')						
	MCF-7	PC3	DU145**	RAJI			
	Inactivator dose (10 µM)			Inactivator dose (µM) 10	1.0	0.5	0.1
B4311	---	5.56	3.75	73.3	8.25	---	1.4
B4314*	---	2.0	1.71	84.0	4.61	---	3.46
B4316	8.0	7.6	3.53	66	13.2	---	1.4
B4349	4.8	3.6	4.0	50.8	33.0	---	2.4
BeG	2.94	2.88	5.45	27.5	1.89	---	1.03
PaTrin-2	3.13	4.6	4.14	60	33.0	8.0	5.5

* Toxic to Raji cells at 10 µM

** Sensitisation factor = D_{60} control/D_{60} 'B'

--- Not done

TABLE 5

EFFECT OF INACTIVATOR PRETREATMENT ON SENSITISATION OF VARIOUS HUMAN CANCER CELL LINES TO BCNU

INACTIVATOR (10 µM)	SENSITISATION FACTOR (D_{50} control/D_{50} 'B')					
	MCF-7	PC3	DU145**	RAJI		
				Inactivator dose (µM) 10	1.0	0.1
B4311	---	1.47	1.56	8.0	---	---
B4314*	---	1.46	1.25	7.62	7.6	3.45
B4316	1.37	1.35	3.57	6.4	---	---
B4349	1.85	1.63	2.78	4.8	---	---
BeG	1.94	1.41	1.79	4.33	---	---
PaTrin-2	1.61	2.11	2.08	6.0	---	---

* Toxic to Raji cells at 10 µM

** Sensitisation factor = D_{60} control/D_{60} 'B'

--- Not done

TABLE 6A

Test No.	O^6-Substituent	Yield % (based on solvate)	Solvent for recrystn.	M.p. (decomp.) (°C)	Formula		Analysis C	H	N
B.4280	4-bromothenyl	73	MeOH	204-205	$C_{10}H_8BrN_5OS$	Found	36.7	2.45	21.46
						Req.	36.82	2.47	21.47
B.4281	5-chlorothenyl[a]	39	MeCN	155-158	$C_{10}H_8ClN_5OS$	Found	41.81	2.86	24.10
						Req.	42.63	2.86	24.86
B.4283	5-cyanothenyl[b]	10	MeOH	200 upwards	$C_{11}H_8N_6OS.$ 0.5 H_2O	Found	47.01	2.94	28.24
						Req.	46.97	3.23	29.88
B.4294	5-methylsulph-inylthenyl	32	MeOH	200 upwards	$C_{11}H_{11}N_5O_2S_2$	Found	42.58	3.62	22.27
						Req	42.71	3.58	22.64
B.4298	4-chlorothenyl	34	MeCN	194-198	$C_{10}H_8ClN_5OS$	Found	42.70	2.94	24.84
						Req	42.63	2.86	24.86
B.4300	4-methoxythenyl	44	MeOH	189-190	$C_{11}H_{11}N_5O_2S$	Found	47.73	4.15	25.05
						Req	47.64	4.00	25.26
B.4313	5-bromo-3-thienylmethyl	7.6	MeCN	190 upwards	$C_{10}H_8BrN_5OS$	Found	37.02	2.43	20.95
						Req	36.82	2.47	21.47
B.4317	4-cyanothenyl	32	MeOH	213-216	$C_{11}H_8N_6OS$	Found	48.50	2.84	30.66
						Req	48.52	2.96	30.87
B.4318	4,5-dichlorothenyl	38	MeOH	210 upwards	$C_{10}H_7Cl_2N_5OS.$ $1H_2O$	Found	35.94	2.67	20.96
						Req	35.94	2.71	20.96
B.4321	2-chloro-4-picolyl	10	MeOH	234 upwards	$C_{11}H_9ClN_6O$	Found	47.15	3.52	29.32
						Req	47.75	3.29	30.37
B.4336	5-bromofurfuryl	39	MeOH	180 upwards	$C_{10}H_8BrN_5O_2.$ 0.25 H_2O	Found	38.22	2.71	21.93
						Req	38.18	2.72	22.26

[a] 5.6 mmol alcohol per mmol quaternary salt used in synthesis. [b] Dimethylformamide reaction solvent.

O^6-Substituted guanines (continuation of Table 6a)

Compound, Test No.	O^6-Substituted RCH_2	Yield %	Solvent for Recrystn.	M.p. (decomp) (C°)	Formula	Molecular Weight	Analysis	C	H	N
B.4282	3-picolyl *N*-oxide	54	MeOH	244-254	$C_{11}H_{10}N_6O_2$	258				
B.4309	5-methylsulphonyl-thenyl	12	EtOH	206-209	$C_{11}H_{11}N_5O_3S_2$.½ C_2H_5OH	348	Found Req.	41.46 41.37	3.83 4.05	20.13 20.10
B.4319	6-chloro-3-picolyl	58	MeOH	> 215	$C_{11}H_9Cl\,N_6O$.½ H_2O	285.7	Found Req.	46.01 46.25	3.49 3.53	29.05 29.42
B.4320	5-bromo-3-picolyl	56	MeOH	> 220	$C_{11}H_9BrN_6O$.½ H_2O	330	Found Req.	40.02 39.87	3.05 3.01	25.28 25.28
B.4354	4-isothiazolyl	28	MeOH	> 200	$C_9H_8N_6OS$.¾ H_2O	261.8	Found Req.	41.59 41.32	3.64 3.59	31.53 32.16
B.4356	4-methylthiothenyl	30	MeOH		$C_{11}H_{11}N_5OS_2$	293.4				
B.4357	5-iodo-3-thienyl-methyl	23	MeOH	> 200	$C_{10}H_8I\,N_5OS$	373				
B.4361	4-methyl-sulphonylthenyl	95	MeOH	170-172	$C_{11}H_{11}N_5O_3S_2$	325	Found Req.	40.2 40.61	3.39 3.41	21.01 21.53
B.4366	naphtho[2,1-*b*]-thiophen-2-yl-methyl	81	MeOH	> 150	$C_{18}H_{13}N_5OS$	347				
B.4373	4-azidothenyl	37	MeOH	> 195°	$C_{10}H_8N_8SO$	288				
B.4377	4-methyl-sulphinylthenyl	55	MeOH	204-206	$C_{11}H_{11}N_5O_2S_2$	309				
B.4378	5-phenylthenyl	54	CH_3CN	>170	$C_{16}H_{13}N_5OS$	323				

TABLE 6B

Test No.	O^6-Substituent	λ_{max} (nm)(MeOH)	δ_H [ppm from TMS; $(CD_3)_2SO$], J (Hz)
B. 4280	4-bromothenyl	238, 284 (RCH_2OH:233).	5.65(s), 6.40(s), 7.37(d), 7.71(d), 7.85(s), 12.49(s).
B. 4281	5-chlorothenyl	247, 284 (RCH_2OH:245).	5.59(s), 6.40(s), 7.06(d), 7.22(d), 7.87(s), 12.47(bs).
B. 4283	5-cyanothenyl	247, 272	5.73(s), 6.46(s), 7.49(d), 7.87(s), 7.92(d), 12.54(bs).
B. 4294	5-methylsulphinylthenyl	243, 284(sh) [RCH_2OH:240, 274(sh)].	2.93(s), 5.73(s), 6.41(s), 7.40(d), 7.52(d), 7.88(s), 12.52(bs).
B. 4298	4-chlorothenyl	238, 284 (RCH_2OH:240).	5.64(s), 6.42(s), 7.34(d), 7.62(d), 7.86(s), 12.51(s).
B. 4300	4-methoxythenyl	245(sh), 282 (RCH_2OH: 258).	3.75(s), 5.57(s), 6.37(s), 6.60(d), 7.01(d), 7.85(s), 12.48(s).
B. 4313	5-bromo-3-thienylmethyl	240, 284 (RCH_2OH: 236).	5.42(s), 6.38(s), 7.40(d), 7.72(d), 7.85(s), 12.47(s).
B. 4317	4-cyanothenyl	244, 284 (RCH_2OH: 244).	5.68(s), 6.44(s), 7.74(d), 7.86(s), 8.60(d), 12.50(s).
B. 4318	4,5-dichlorothenyl	243, 285 (RCH_2OH:243).	5.58(s), 6.45(s), 7.41(s), 7.87(s), 12.52(s).
B. 4321	2-chloro-4-picolyl	241, 272(sh), 285 [RCH_2OH: 262, 268(sh)].	5.58(s), 6.36(s), 7.51(bs), 7.61(bs), 7.91(bs), 8.44(bs), 12.56(bs).
B. 4336	5-bromofurfuryl	220, 284 (RCH_2OH: 223)	5.42(s), 6.39(s), 6.64(d), 6.78(d), 7.85(s), 12.49(s).

O^6-Substituted guanines (continuation of Table 6B)

Compound Type, Test No.	O^6-Substituent RCH_2	λ_{max} (MeOH) (nm)	δ_H[ppm from TMS, $(CD_3)_2SO$,]J(Hz)
B.4282	3-picolyl *N*-oxide	271	5.48(s), 6.41(s), 7.47(m), 7.87(s), 8.22(m), 8.42(s), 12.52(s).
B.4309	5-methylsulphonyl-thenyl	242, 284	5.75(s), 6.43(s), 7.47(d), 7.74(d), 7.87(s), 12.52(s).
B.4319	6-chloro-3-picolyl	242, 276	5.53(s), 6.38(s), 7.59(d), 7.87(s), 8.05(dd), 8.64(d), 12.48(s).
B.4320	5-bromo-3-picolyl	242, 281	5.53(s), 6.41(s), 7.86(s), 7.86(s), 8.26(dd), 8.73(d), 8.78(d), 12.50(s).
B.4354	4-isothiazolyl	244, 284	5.58(s), 6.41(s), 7.84(s), 8.81(s), 9.22(s), 12.47(s).
B.4356	4-methylthio-thenyl	236, 283	2.48(s), 5.62(s), 6.40(s), 7.26(m), 7.85(s), 12.48(s).
B.4357	5-iodo-3-thienylmethyl	240, 283	5.43(s), 6.38(s), 7.48(d), 7.77(s), 7.84(s), 12.47(s).
B.4361	4-methylsulphonyl-thenyl	240, 285	3.26(s), 5.70(s), 6.40(s), 7.72(s), 7.85(s), 8.38(d), 12.49(s).
B.4366	naphtho[2,1-*b*]-thiophen-2-ylmethyl	244, 286sh 295, 306sh	5.90(s), 6.47(s), 7.60(t), 7.69(t), 7.86(t), 8.04(dd), 8.44(s), 8.51(d), 12.51(s).
B.4373	4-azidothenyl	227, 280	5.64(s), 6.36(s), 7.20(s), 7.28(s), 7.84(s), 12.47(s).
B.4377	4-methylsulphinyl-thenyl	241, 285	2.82(s), 5.68(s), 6.33(s), 7.60(s), 7.82(s), 8.01(s), 12.45(s).
B.4378	5-phenylthenyl	244sh, 289	5.67(s), 6.32(s), 7.31(m), 7.41(m), 7.41(m), 7.63(d), 7.82(s), 12.43(s).

TABLE 7

INACTIVATOR	M.Wt	I_{50}(μM) hAT	Raji I_{50} (μM)	Stability T 1/2(h) By Spec
B.4280 Q^6-(4-bromothenyl)guanine	326	0.0034		
B.4281 Q^6-(5-chlorothenyl)guanine	281.7	0.004		>10
B.4282 Q^6-(oxido-3-picolyl)guanine	276	1.4		>20
B.4283 Q^6-(5-cyanothenyl)guanine	272	0.005		>20
B.4294 Q^6-(5-methylsulphinylthenyl)guanine	309	0.03		>10
B.4298 Q^6-(4-chlorothenyl)guanine	282	0.008	0.005	>16
B.4300 Q^6-(4-methoxythenyl)guanine	277	0.0165		0.83

TABLE 7 (continued)

INACTIVATOR	M.Wt	I_{50}(μM) hAT	Raji I_{50} (μM)	Stability T 1/2 (h) By Spec
B.4309 O^6-(5-methylsulphonylthenyl)guanine	325	0.072		>16
B.4313 O^6-(5-bromo-3-thienylmethyl)guanine	326	0.0065	0.035	
B.4317 O^6-(4-cyanothenyl)guanine	272	0.0028		>19
B.4318 O^6-(4,5-dichlorothenyl)guanine	348	0.015		2.5
B.4319 O^6-(6-chloro-3-picolyl)guanine	277	0.2		>13
B.4320 O^6-(5-bromo-3-picolyl)guanine	321	0.25		>13
B.4321 O^6-(2-chloro-4-picolyl)guanine	277	0.04		>16

TABLE 7 (continued)

INACTIVATOR	M.Wt	I_{50}(μM) hAT	Raji I_{50} (μM)	Stability T 1/2 (h) By Spec
B.4336 O^6-(5-bromofurfuryl)guanine	310	0.02		0.32
B.4354 O^6-(4-isothiazolylmethyl)guanine	248	0.07		
B.4356 O^6-(4-methylthiothenyl)guanine	293	0.0095		
B.4357 O^6-(5-iodo-3-thienylmethyl)guanine	447	0.009		>16
B.4361 O^6-(4-methylsulphonylthenyl)guanine	325	0.2		>16
B.4366 O^6-(naphtho[2,1-b]thiophen-2-ylmethyl)guanine	347	0.05		

TABLE 7 (continued)

INACTIVATOR	M.Wt	I_{50}(μM) hAT	Raji I_{50} (μM)	Stability T 1/2 (h) By Spec
B.4368 9-(B-D-arabinofuranosyl)-O^6-(4-bromothenyl)guanine	458	0.115		
B.4369 O^6-(4-bromothenyl)-9-(ethoxymethyl)guanine	384	0.28		
B.4370 O^6-(4-bromothenyl)-9(octyloxymethyl)guanine	468	1.2		
B.4373 O^6-(4-azidothenyl)guanine	288	0.0063		
B.4377 O^6-(4-methylsulphinylthenyl)guanine	309	0.15		
B.4378 O^6(5-phenylthenyl)guanine	323	0.75		
B.4379 O^6-(4-bromothenyl)-2-deoxyguanosine	442	0.095		

TABLE 7B

INACTIVATOR	M.Wt	In vitro I_{50} (μM)					Raji I_{50} (μM)	Stability T 1/2 (h)	
		hAT	mAT	rAT	chAT	ogt		By Spec	By Assay
B.4363 O^6-(4-bromothenyl)guanosine	458	0.08		0.24	0.95	30		>16	>48

Blank space = not done

TABLE 8

ATASE ACTIVITY IN VARIOUS TISSUES OF NU/NU MICE AFTER TREATMENT WITH 10mg/kg (IP) B.4280

Tissue	MEAN ACTIVITY (fm/mg) 24h	48h	Control*
Tumour	36 ± 7.79	140 ± 43.87	125
Liver	89.7 ± 10.14	100.7 ± 8.73	110**
Lung	15.3 ± 2.05	24 ± 2.83	43
Kidney	24.3 ± 4.03	28.7 ± 4.11	33
Spleen	41 ± 5.35	68.3 ± 9.53	81
Brain	13.7 ± 2.05	16.3 ± 1.25	14
Testis	45 ± 7.48	44 ± 1.41	45
Bone Marrow (pooled)	42	61	30

* control values taken from a separate experiment
** mean of 2 control liver values

Table 8.
Effect of B.4280 on ATase activity in several tissues of nude mice. Animals were given a single dose of B.4280 (10mg/kg i.p.) and sacrificed 24 or 48 hours later.

TABLE 9

TOXICITY OF INACTIVATORS IN COMBINATION WITH BCNU IN DBA_2 MICE

INACTIVATOR (60mg/kg)	%SURVIVAL AFTER 14 DAYS 20mg/kg BCNU	16mg/kg BCNU	12mg/kg BCNU
O^6-benzylguanine	33 (2/6)	0 (0/6)*	50 (3/6)**
B.4205	0 (0/6)	50 (3/6)*	100 (6/6)**
B.4280	93 (14/15)	100 (15/15)	100 (15/15)

* 15mg/kg BCNU
** 10mg/kg BCNU

All agents were given as a single i.p. dose

Table 9
Effect of ATase inactivators on the acute toxicity of bis-chloroethylnitrosourea (BCNU) in DBA_2 mice.

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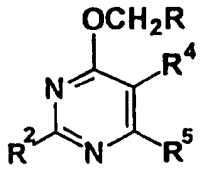
CLAIMS

1. A 6-hetarylalkyloxy pyrimidine derivative of formula II



II

wherein

R is (i) a cyclic group having at least one 5- or 6-membered heterocyclic ring, optionally with a carbocyclic or heterocyclic ring fused thereto, the or each heterocyclic ring having at least one hetero atom chosen from O, N, or S, or a substituted derivative thereof; or (iii) phenyl or a substituted derivative thereof,

R^2 is selected from H, C_1-C_5 alkyl, halogen or NH_2,

R^4 and R^5 which are the same or different are selected from H, NH-Y' or NO_n

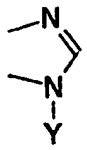
wherein

Y' is H, ribosyl, deoxyribosyl, arabinosyl, R''XCHR''' wherein X is O or S, R'' is alkyl and R''' is H or alkyl, or substituted derivatives thereof,

n = 1 or 2

or R^4 and R^5 together with the pyrimidine ring form a 5 or 6-membered ring structure containing one or more hetero atoms, and pharmaceutically acceptable salts thereof,

with the proviso that R^2 is not NH_2 if R^4 and R^5 form a ring structure IX



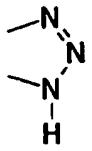
IX

wherein Y is H, ribosyl, deoxyribosyl, or R''XCHR''' wherein X is O or S, R'' and R''' are alkyl, or subsituted derivatives thereof,

and with the proviso that R is not phenyl in the following circumstances a) to h):

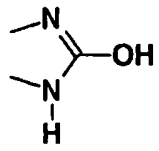
a) if R^2 and R^5 are NH_2 and R^4 is NO or NO_2

b) if R^2 is NH_2 and R^4 and R^5 form a ring structure X



X

c) if R^2 is NH_2 and R^4 and R^5 form a ring structure XI



XI

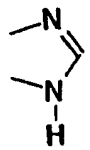
d) if R^2 is NH_2, R^4 is NO_2 and R^5 is H or CH_3

e) if R^2, R^4 and R^5 are NH_2.

f) if R^2 and R^5 are NH_2 and R^4 is H

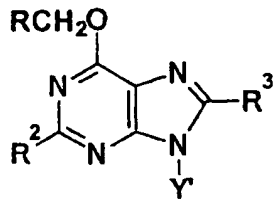
g) if R^2 is H, R^4 is NO_2 and R^5 is NH_2

h) if R^2 is F or OH, and R^4 and R^5 form a ring structure XII



XII

2. A compound according to claim 1 which is of Formula III



III

wherein:

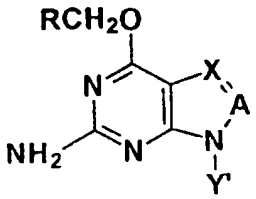
R is as defined in claim 1

Y' is as defined in claim 1;

R^2 is H, NH_2, C_1-C_5 alkyl or halogen;

R^3 is H or OH;

3. A compound according to claim 1 which is of Formula IV



IV

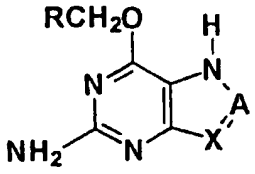
wherein:

R and Y' are as defined in claim 1;

X is CH or N;

A is CH or N;

4. A compound according to claim 1 which is of Formula V



V

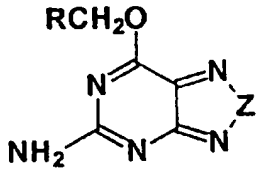
wherein:

R is as defined in claim 1

X is CH or N

A is CH or N.

5. A compound according to claim 1 which is of Formula VI



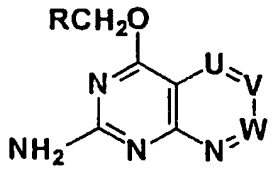
VI

wherein:

R is as defined in claim 1;

Z is O or S or CH = CH.

6. A compound according to claim 1 which is of Formula VII



VII

wherein:

R is as defined in claim 1;

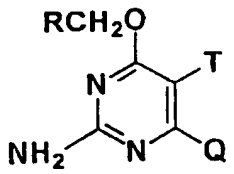
U is CH or N;

V is CH or N;

W is CH or N;

provided that U, V and W are not all CH.

7. A compound according to claim 1 which is of Formula VIII



VIII

wherein:

R is as defined in claim 1

T is H, NH_2 or NO_n where n = 1 or 2;

Q is H, NH_2 or NO_n where n = 1 or 2.

8. A compound according to claim 1, 2 or 3 wherein Y' is H, ribosyl, deoxyribosyl, arabinosyl, $HOCH_2CH_2OCH_2$- or R''XCHR''' wherein X is O or S, R'' and R''' are alkyl, or substituted derivatives thereof.

9. A compound according to claim 1 wherein R is a 5-membered heterocyclic ring, having at least one S atom therein.

10. A compound according to claim 1 wherein R is selected from a thiophene ring, a furan ring, and substituted derivatives thereof.

11. A compound according to claim 1 wherein R includes a heterocyclic and/or carbocyclic ring substituted by halo, haloalkyl, cyano, SO_nR^8 where R^7 is alkyl and n = 0, 1 or 2, or -$COOR^8$ wherein R^8 is alkyl.

12. A compound according to claim 1 wherein R is selected from a thiophene ring, a furan ring and substituted derivatives thereof selected from bromo- and cyano-substituted derivatives thereof.

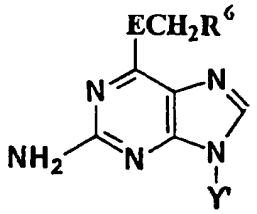
13. A compound according to claim 1 wherein R is selected from thiophene and furan rings with a chloro-, bromo- or cyano-substituent in a 1,3- or 1,4-relationship with the methyleneoxy group attached to the pyrimidine residue.

14. A compound according to claim 1 wherein Y' is alkoxymethyl optionally substituted with OH on the alkyl of the alkoxy group.

15. A compound according to claim 1 which is selected from

Q^6-(4-bromothenyl)-8-thiaguanine.
Q^4-(4-chlorothenyl)pterin.
Q^6-piperonyl -7-deaza-8-azaguanine.
Q^6-(4-bromothenyl)-8-hydroxyguanine.
Q^6-(4-chlorothenyl)-2, 4-diamino-6-hydroxy-5-nitrosopyrimidine.

16. Guanine derivatives of formula XIII



XIII

wherein

E is O or S,

Y' is as defined in claim 1,

R^6 is a cyclic group having at least one 5- or 6-membered heterocyclic ring, optionally with a carbocyclic or heterocyclic ring fused thereto, the or each heterocyclic ring having at least one hereto atom chosen from O, N or S, or a substituted derivative thereof,

and pharmaceutically acceptable salts thereof, with the proviso that compounds published in WO 94/29312 are disclaimed.

17. A compound according to claim 16 wherein R^6 is a 5-membered heterocyclic ring, having at least one S atom therein.

18. A compound according to claim 16 wherein R^6 is selected from a thiophene ring, a furan ring, and substituted derivatives thereof.

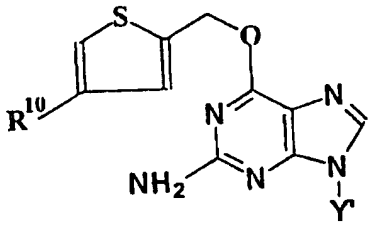
19. A compound according to claim 16 wherein R^6 includes a heterocyclic and/or carbocyclic ring substituted by halo, haloalkyl, cyano, SO_nR^7 where R^7 is alkyl and n = 0, 1 or 2, or $-COOR^8$ wherein R^8 is alkyl.

20. A compound according to claim 16 wherein R^6 is selected from a thiophene ring, a furan ring and substituted derivatives thereof

selected from bromo- and cyano-substituted derivatives thereof.

21. A compound according to claim 16 wherein R^6 is selected from thiophene and furan rings with a chloro-, bromo- or cyano-substituent in a 1,3- or 1,4-relationship with the methyleneoxy group attached to the pyrimidine residue.

22. Guanine derivatives of formula XIV:



XIV

wherein R^{10} is bromo, chloro or cyano, and Y' is as defined in claim 1.

23. A compound according to claim 22 wherein Y' is H, ribosyl, deoxyribosyl, or R''XCHR''' wherein X is O or S, R'' is alkyl and R''' is H or alkyl, or substituted derivatives thereof.

24. Q^6-(4-bromothenyl)guanine.

25. A compound according to claim 22 which is Q^6-(4-bromothenyl)-9-(2-hydroxyethoxymethyl)guanine.

26. A compound according to claim 22 which is 9(B-D-arabinofuranosyl)-Q^6-(4-bromothenyl)guanine.

27. A compound according to claim 22 which is Q^6-(4-bromothenyl)guanosine.

28. A compound according to claim 22 which is Q^6-(4-bromothenyl)-2-deoxyguanosine.

29. A compound according to claim 20 which is selected from:

O^6-(5-chlorothenyl)guanine

O^6-(5-cyanothenyl)guanine

O^6-(5-methylsulphinylthenyl)guanine

O^6-(4-chlorothenyl)guanine

O^6-(4-methoxythenyl)guanine

O^6-(5-bromo-3-thienylmethyl)guanine

O^6-(4-cyanothenyl)guanine

O^6-(4,5-dichlorothenyl)guanine

30. A compound according to claim 20 which is selected from

O^6-(4-methylthiothenyl)guanine

O^6-(4-azidothenyl)guanine.

31. A compound according to claim 16 which is selected from

O^6-(2-chloro-4-picolyl)guanine

O^6-(5-bromofurfuryl)guanine

32. A pharmaceutical composition comprising a compound according to any of claims 1 to 31 and a pharmaceutically acceptable excipient.

33. A pharmaceutical composition according to claim 32 further comprising an alkylating agent.

34. A composition according to claim 33 wherein the alkylating agent is selected from 1,3 bis (2-chloroethyl)-1-nitrosourea (BCNU)

and temozolomide.

35. A method for depleting O^6-alkylguanine-DNA alkyltransferase activity in a host comprising:

administering to the host an effective O^6-alkylguanine-DNA alkyltransferase activity depleting amount of a composition comprising a compound according to any of claims 1 to 31.

36. A method for treating tumour cells in a host comprising:

administering to the host a composition comprising an inactivator compound according to any of claims 1 to 31 in an amount effective to deplete O^6-alkylguanine -DNA alkyltransferase activity sufficiently to enhance the effectiveness of a chemotherapeutic akylating agent; and

administering to the host a composition comprising an alkylating agent in an amount which is cytotoxically effective in combination with the said inactivator compound.

37. Use of a compound according to any of claims 1 to 31 in the manufacture of a medicament for depleting O^6-alkylguanine-DNA alkyltransferase activity in tumour cells.

38. A pharmaceutical composition comprising O^6-(4-bromothenyl)guanine and a pharmaceutically acceptable excipient.

39. A pharmaceutical composition according to claim 38 which is suitable for oral administration.

40. A pharmaceutical composition according to claim 38 further comprising an alkylating agent.

41. A pharmaceutical composition according to claim 40 wherein the

akylating agent is selected from 1,3 bis(2-chloroethyl)-1-nitrosourea (BCNU) and temozolomide.

42. A method for depleting O^6-alkylguanine -DNA alkyltransferase activity in a host comprising:

administering to the host an effective O^6-alkylguanine-DNA alkyltransferase activity depleting amount of a composition comprising O^6-(4-bromothenyl)guanine.

43. A method for treating tumour cells in a host comprising:

administering to the host a composition comprising O^6(4-bromothenyl)guanine in an amount effective to deplete O^6-alkylguanine-DNA alkyltransferase activity sufficiently to enhance the effectiveness of a chemotherapeutic alkylating agent; and

administering to the host a composition comprising an alkylating agent in an amount which is cytotoxically effective in combination with O^6-(4-bromothenyl)guanine.

44. O^6-(methylene[^{3}H]-(4-bromothenyl)guanine.

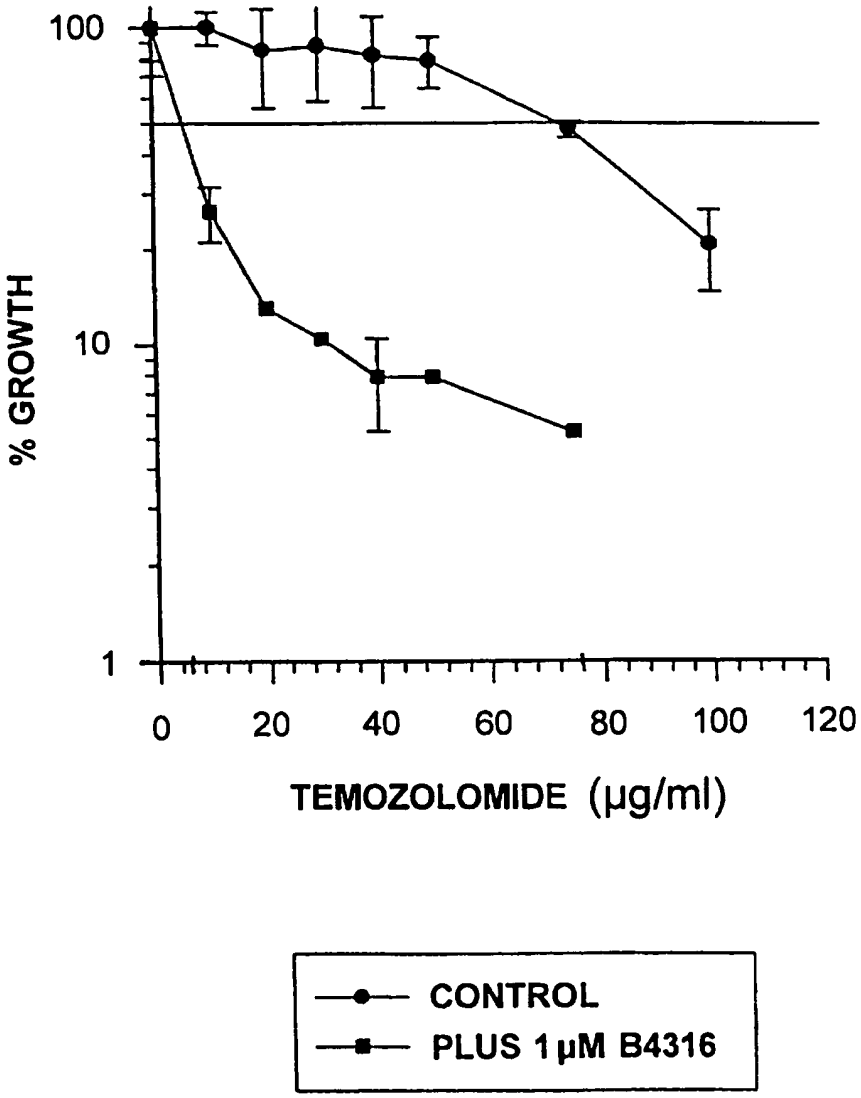

100
10
1
% GROWTH
0
20
40
60
80
100
120
TEMOZOLOMIDE (µg/ml)
CONTROL
PLUS 1 µM B4316

FIG. 1

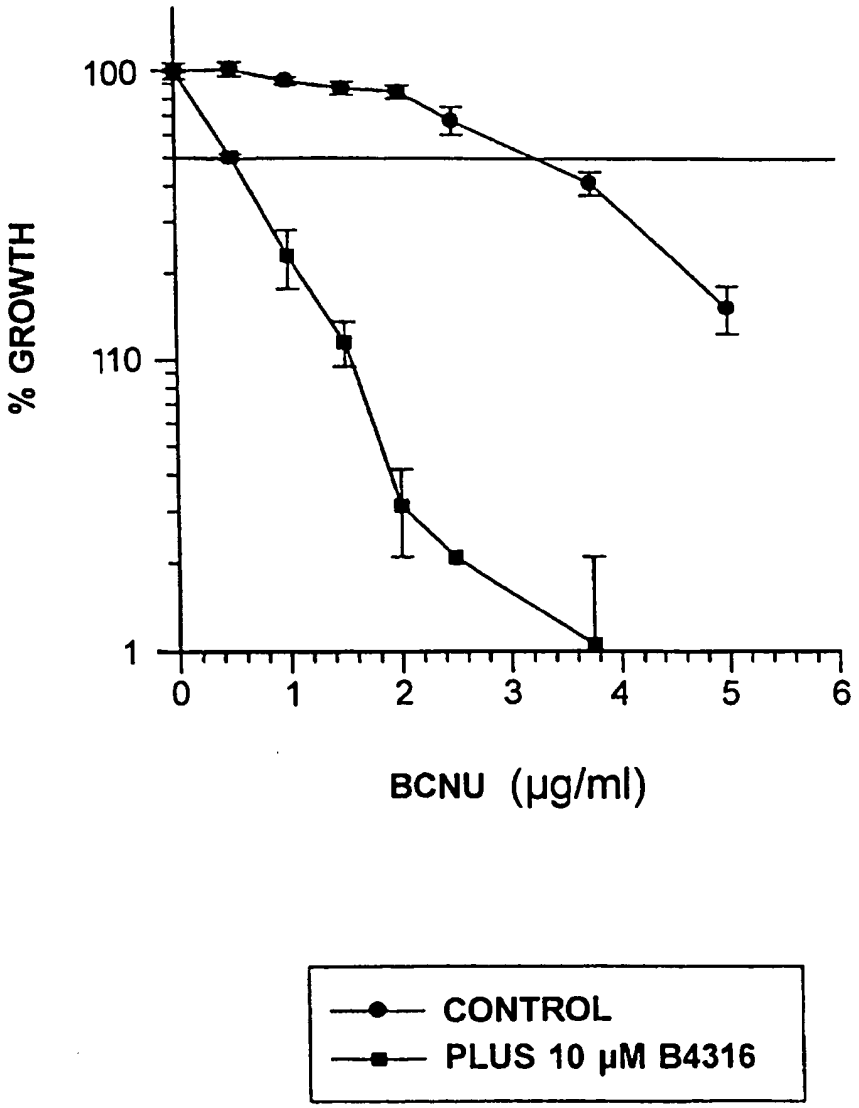


FIG. 2

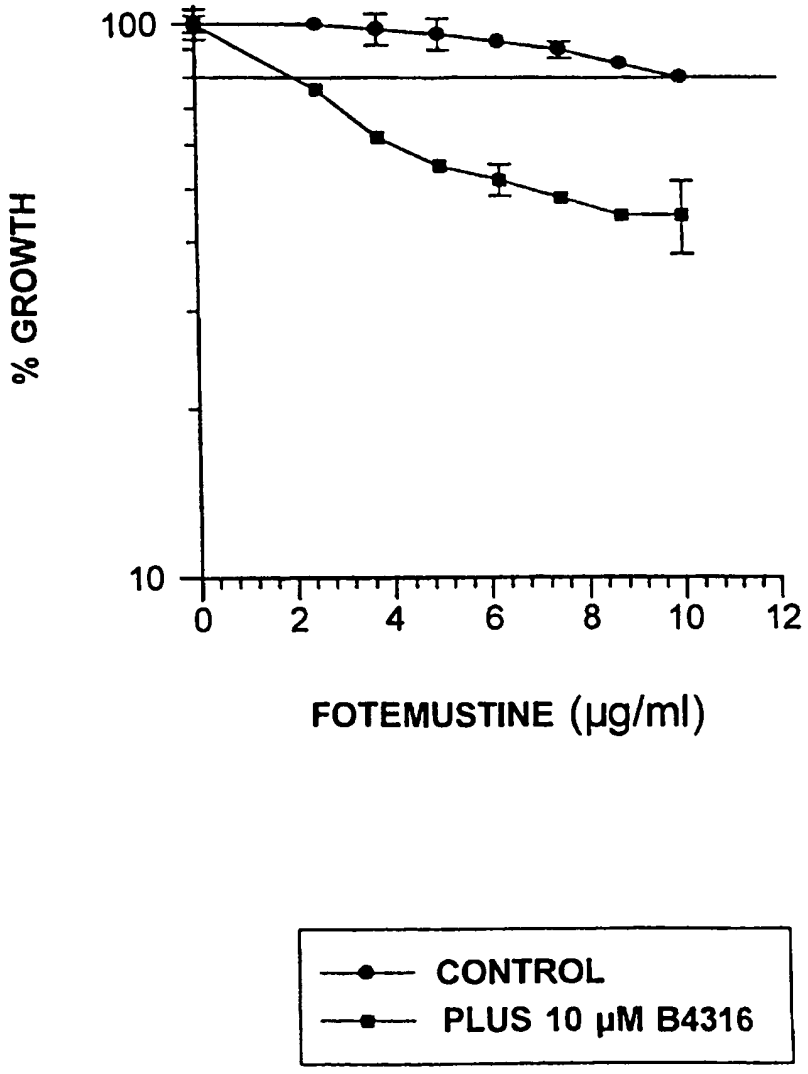

100
10
% GROWTH
0
2
4
6
8
10
12
FOTEMUSTINE (µg/ml)
CONTROL
PLUS 10 µM B4316

FIG. 3

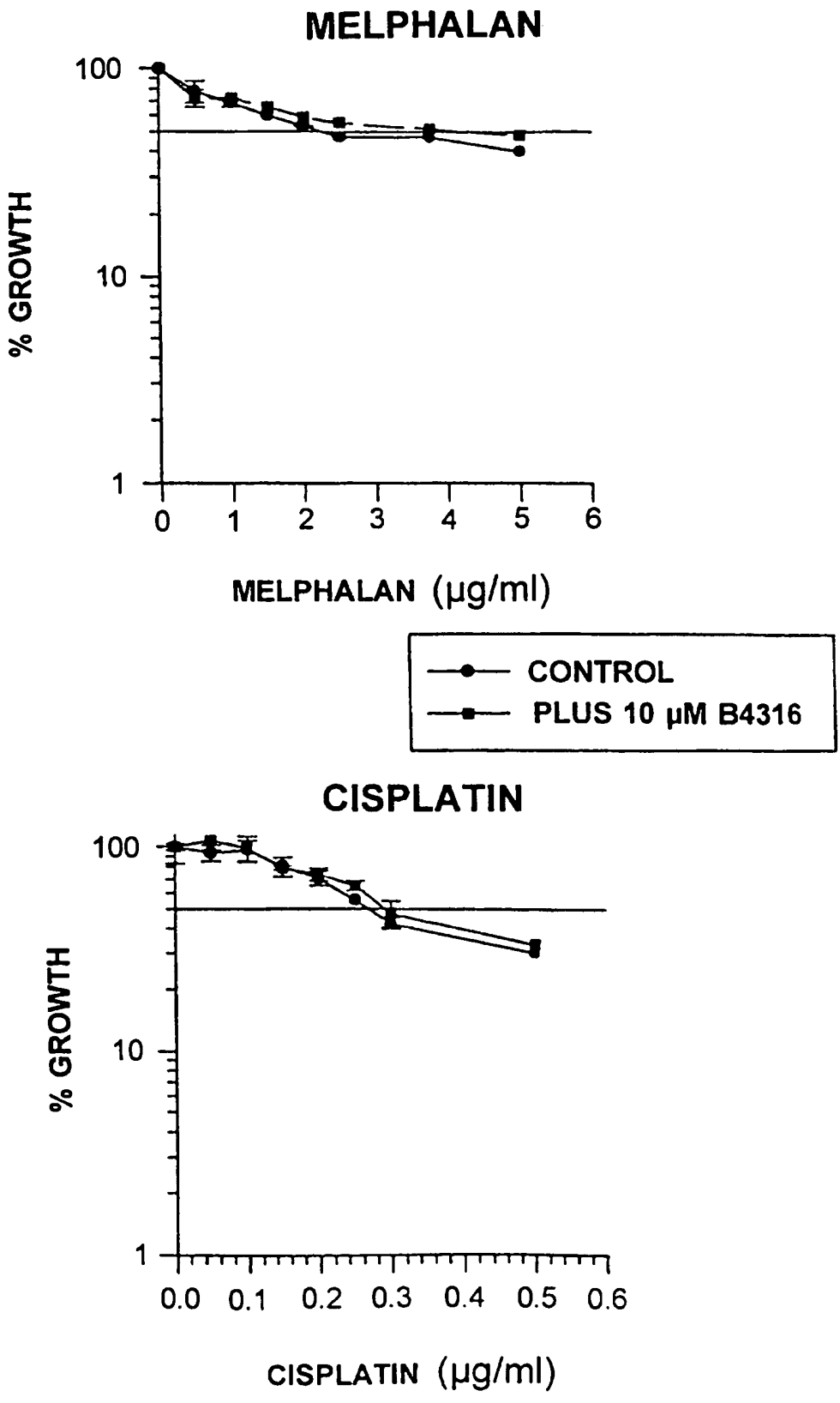
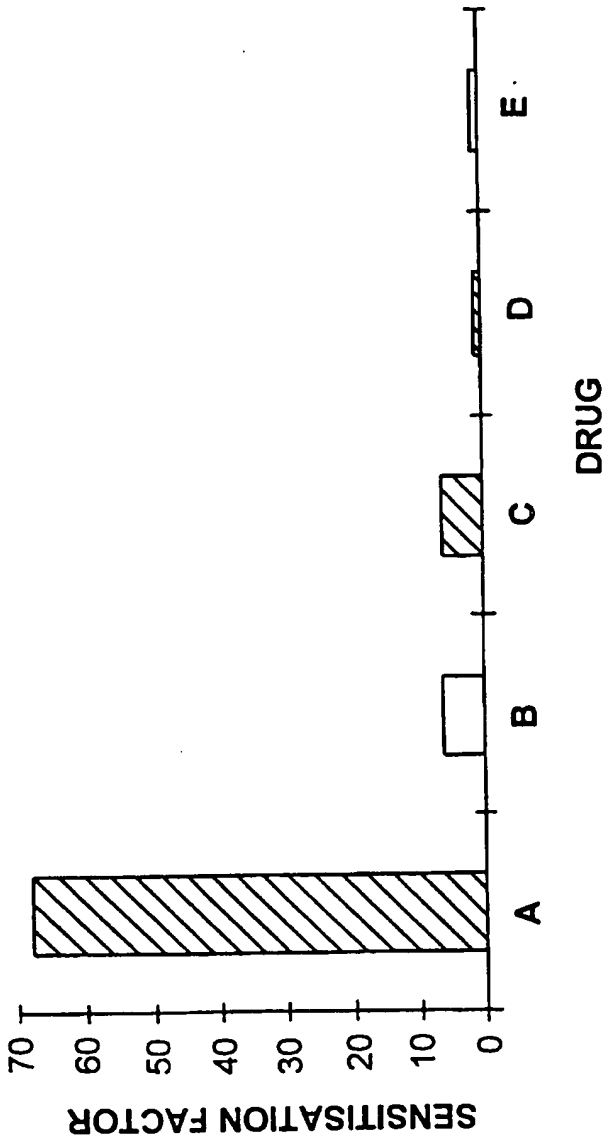
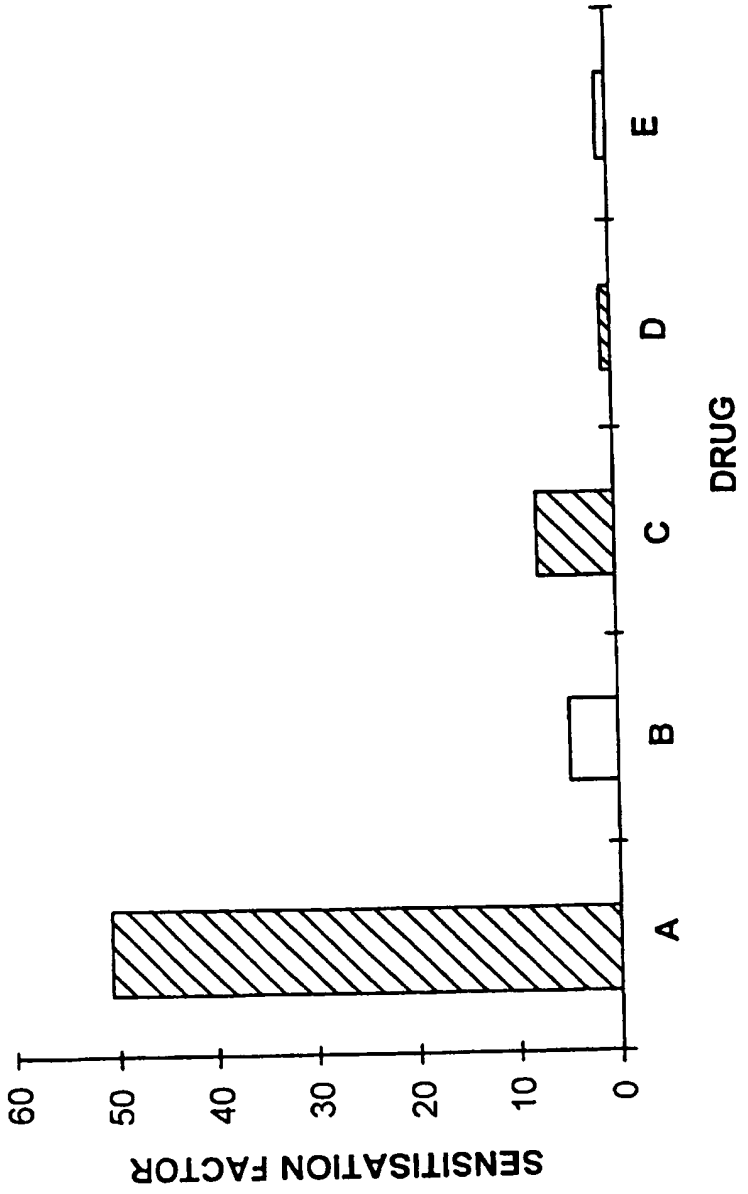

MELPHALAN
100
10
1
% GROWTH
0
1
2
3
4
5
6
MELPHALAN (µg/ml)
CONTROL
PLUS 10 µM B4316
CISPLATIN
100
10
1
% GROWTH
0.0
0.1
0.2
0.3
0.4
0.5
0.6
CISPLATIN (µg/ml)

FIG. 4



	DRUG	S.F.
A	TEMOZOLOMIDE	67.7
B	BCNU	6.4
C	FOTEMUSTINE	6.25
D	MELPHALAN	1
E	CISPLATIN	1

FIG. 5



	DRUG	S.F.
A	TEMOZOLOMIDE	50.7
B	BCNU	4.8
C	FOTEMUSTINE	7.7
D	MELPHALAN	1
E	CISPLATIN	1

FIG. 6

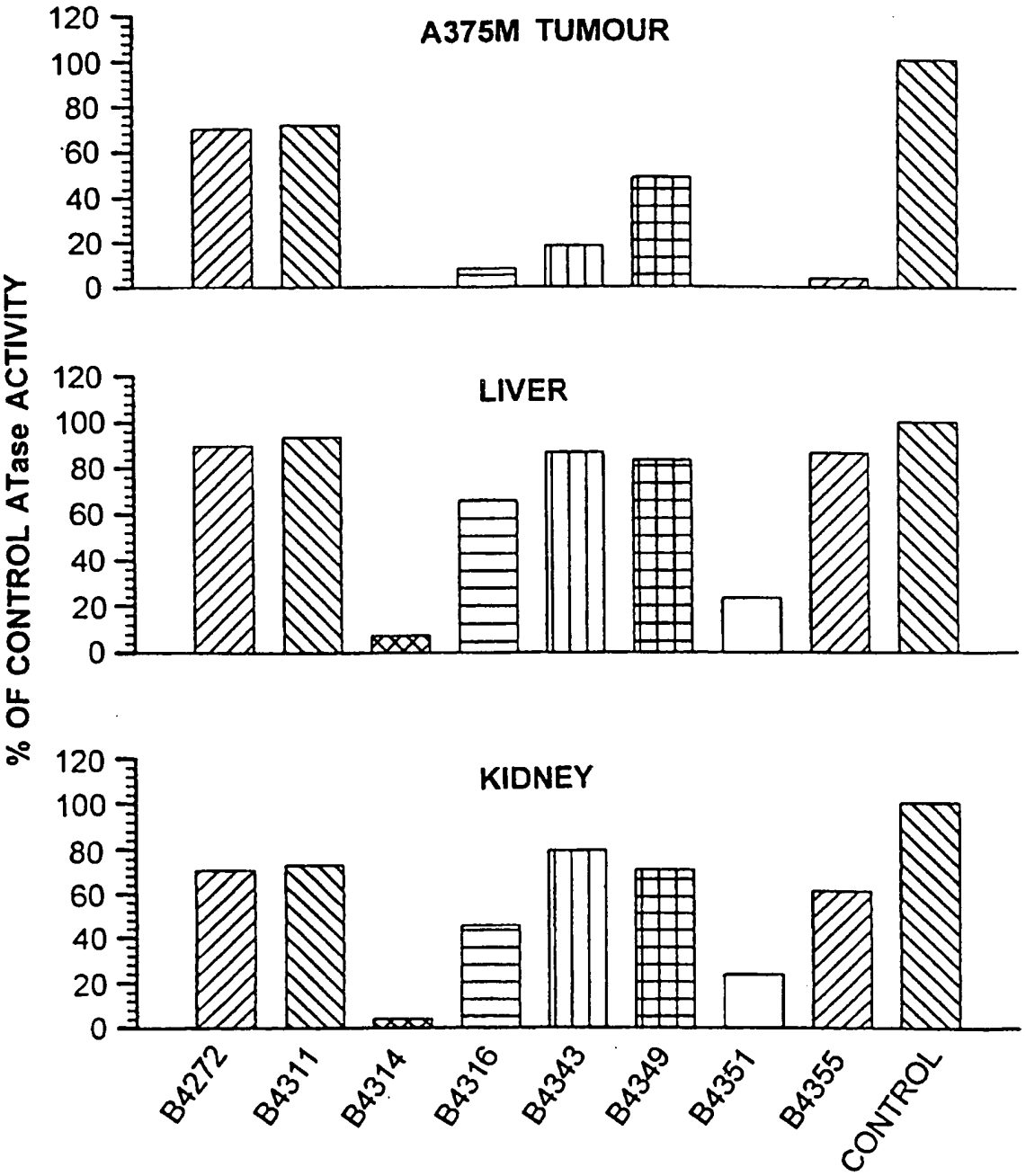
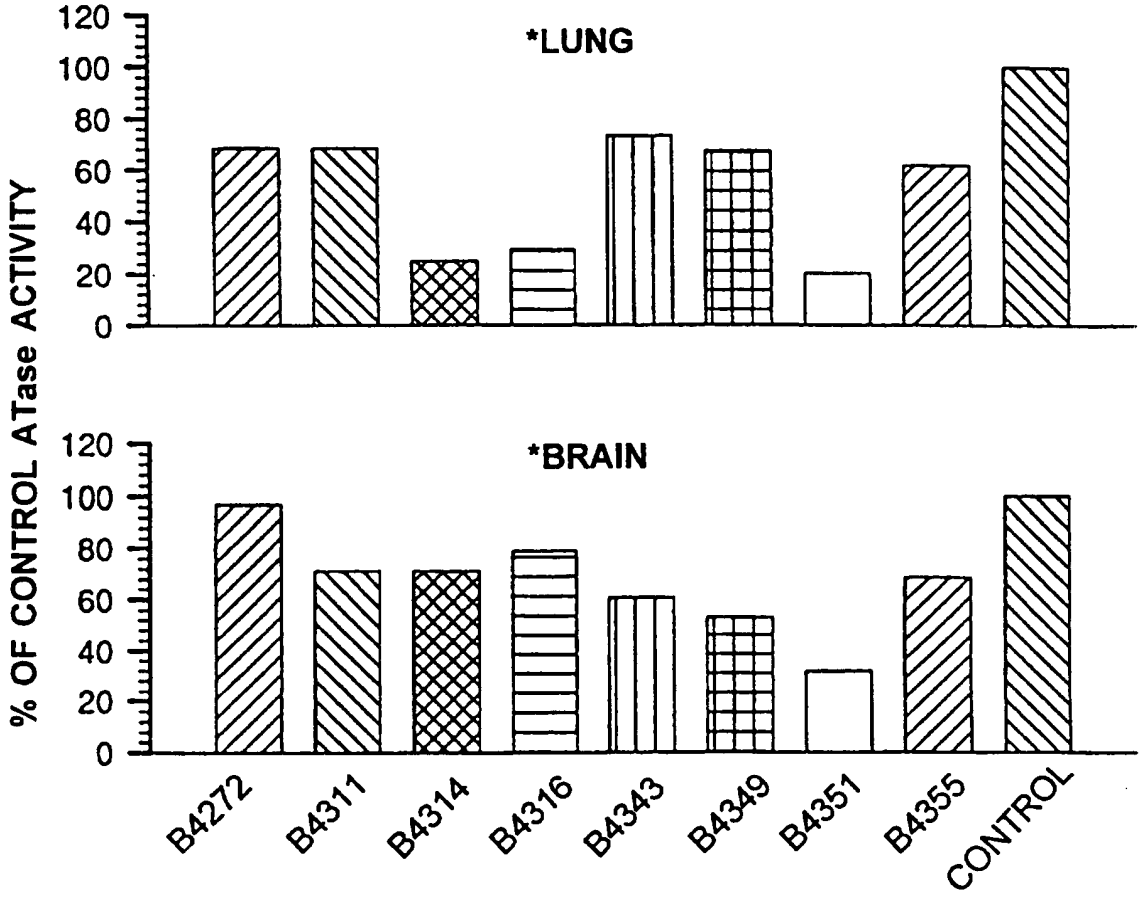

A375M TUMOUR
LIVER
KIDNEY
% OF CONTROL ATase ACTIVITY
120
100
80
60
40
20
0
B4272
B4311
B4314
B4316
B4343
B4349
B4351
B4355
CONTROL

FIG. 7A



*HISTORICAL CONTROLS USED

FIG. 7B

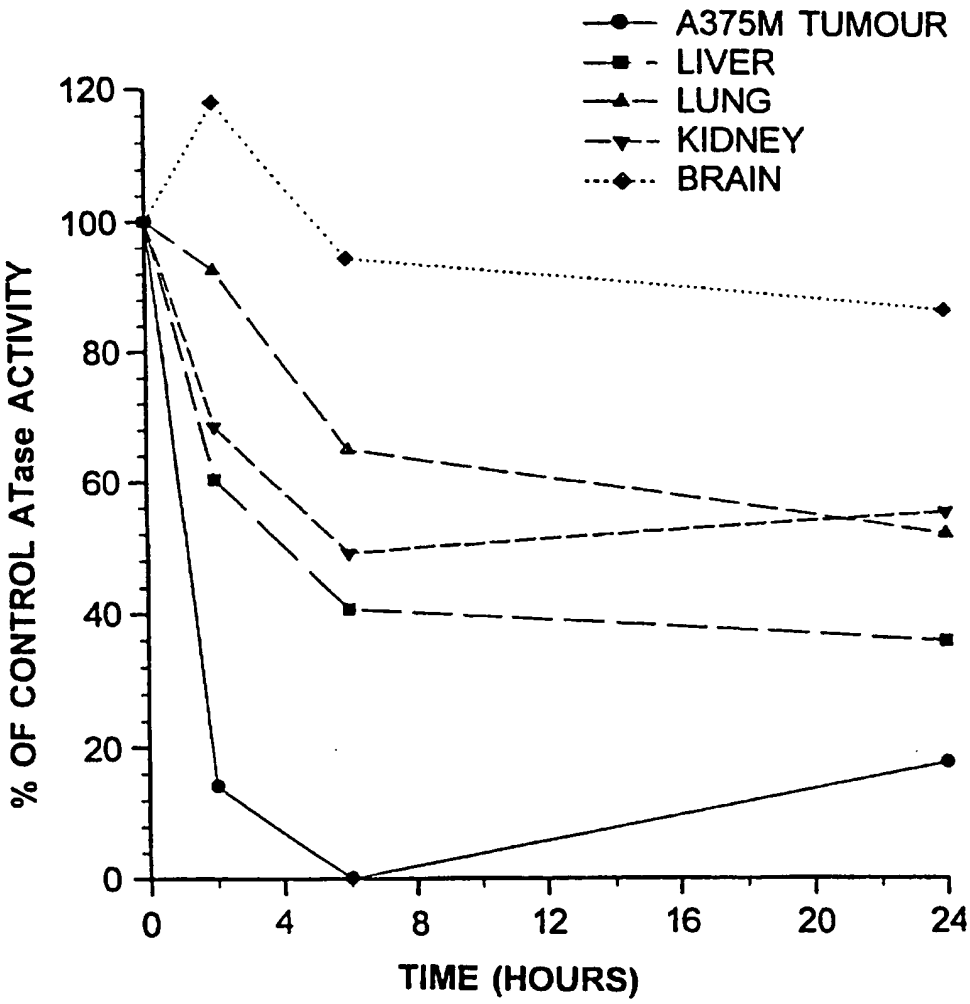

A375M TUMOUR
LIVER
LUNG
KIDNEY
BRAIN
% OF CONTROL ATase ACTIVITY
120
100
80
60
40
20
0
0
4
8
12
16
20
24
TIME (HOURS)

FIG. 8

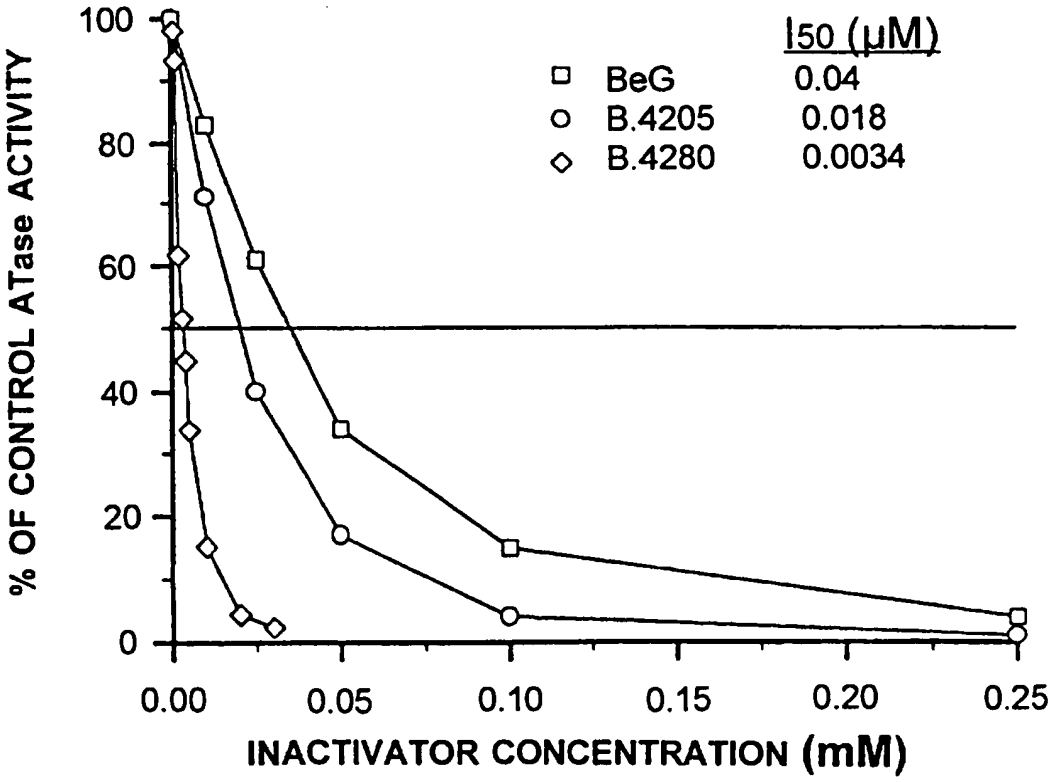
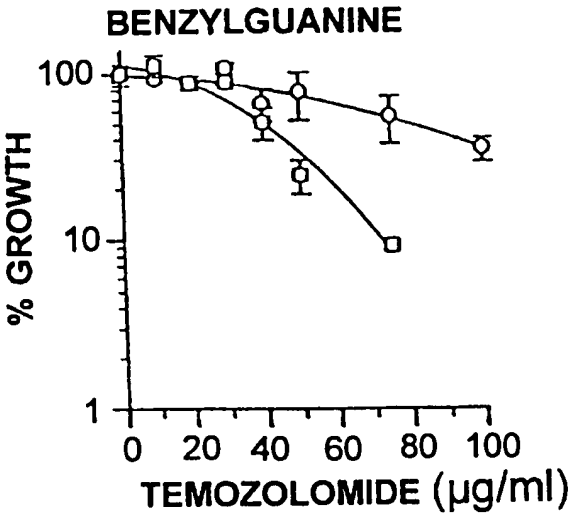
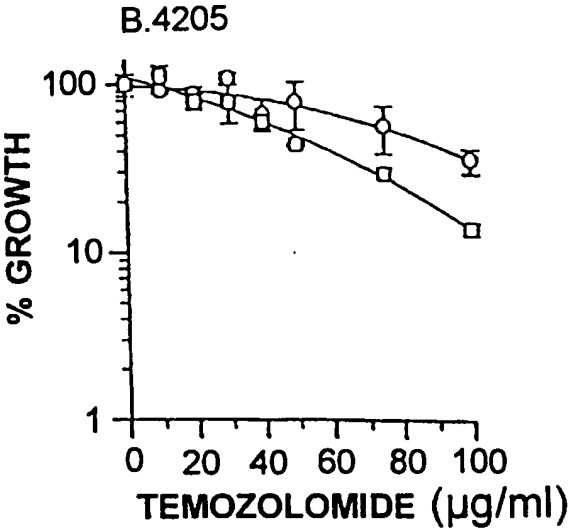

% OF CONTROL ATase ACTIVITY
100
80
60
40
20
0
0.00
0.05
0.10
0.15
0.20
0.25
INACTIVATOR CONCENTRATION (mM)
I50 (μM)
BeG 0.04
B.4205 0.018
B.4280 0.0034

FIG. 9





○ CONTROL

□ 0.5µM INACTIVATOR

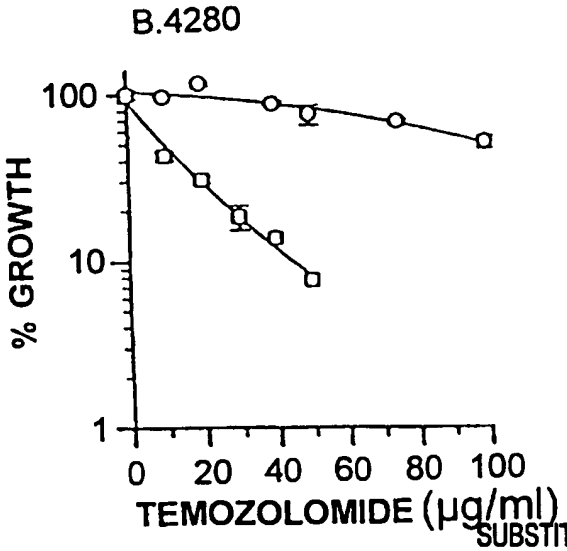


FIG. 10A

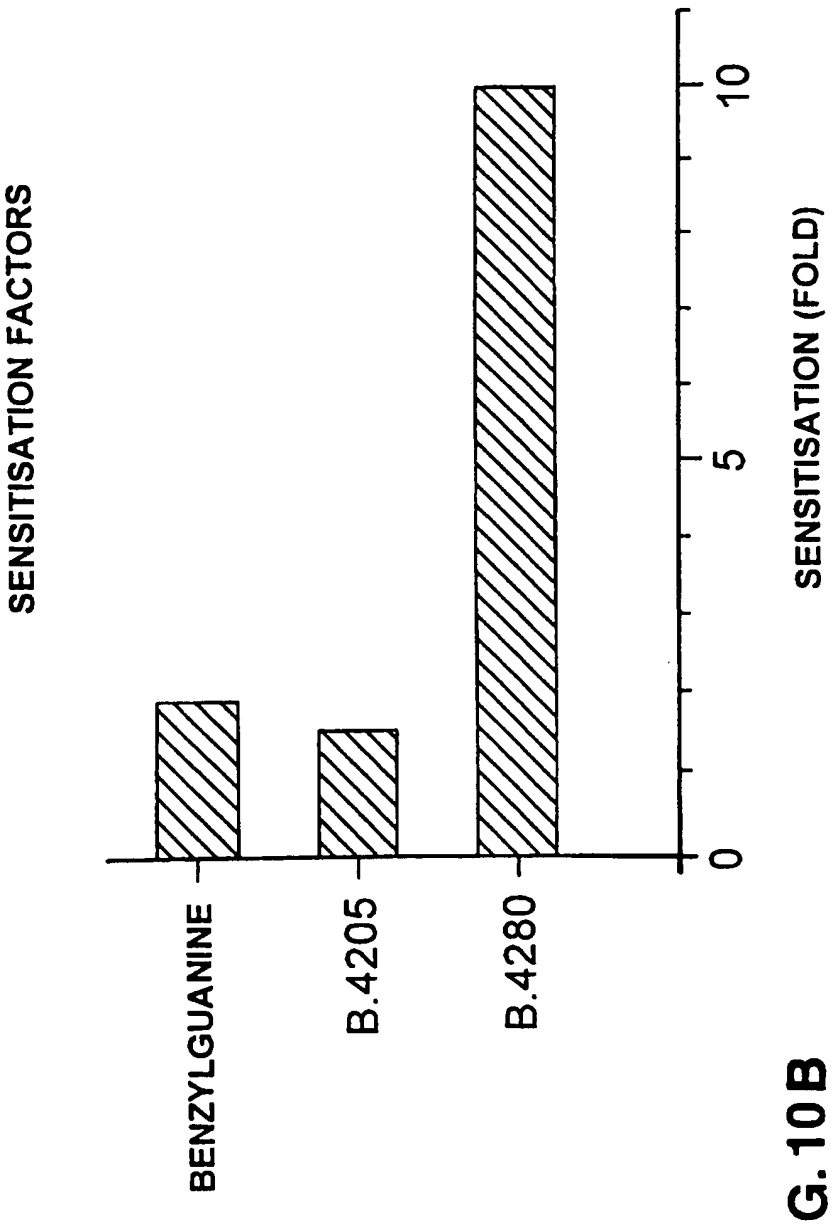

SENSITISATION FACTORS
BENZYLGUANINE
B.4205
B.4280
0
5
10
SENSITISATION (FOLD)

FIG. 10B

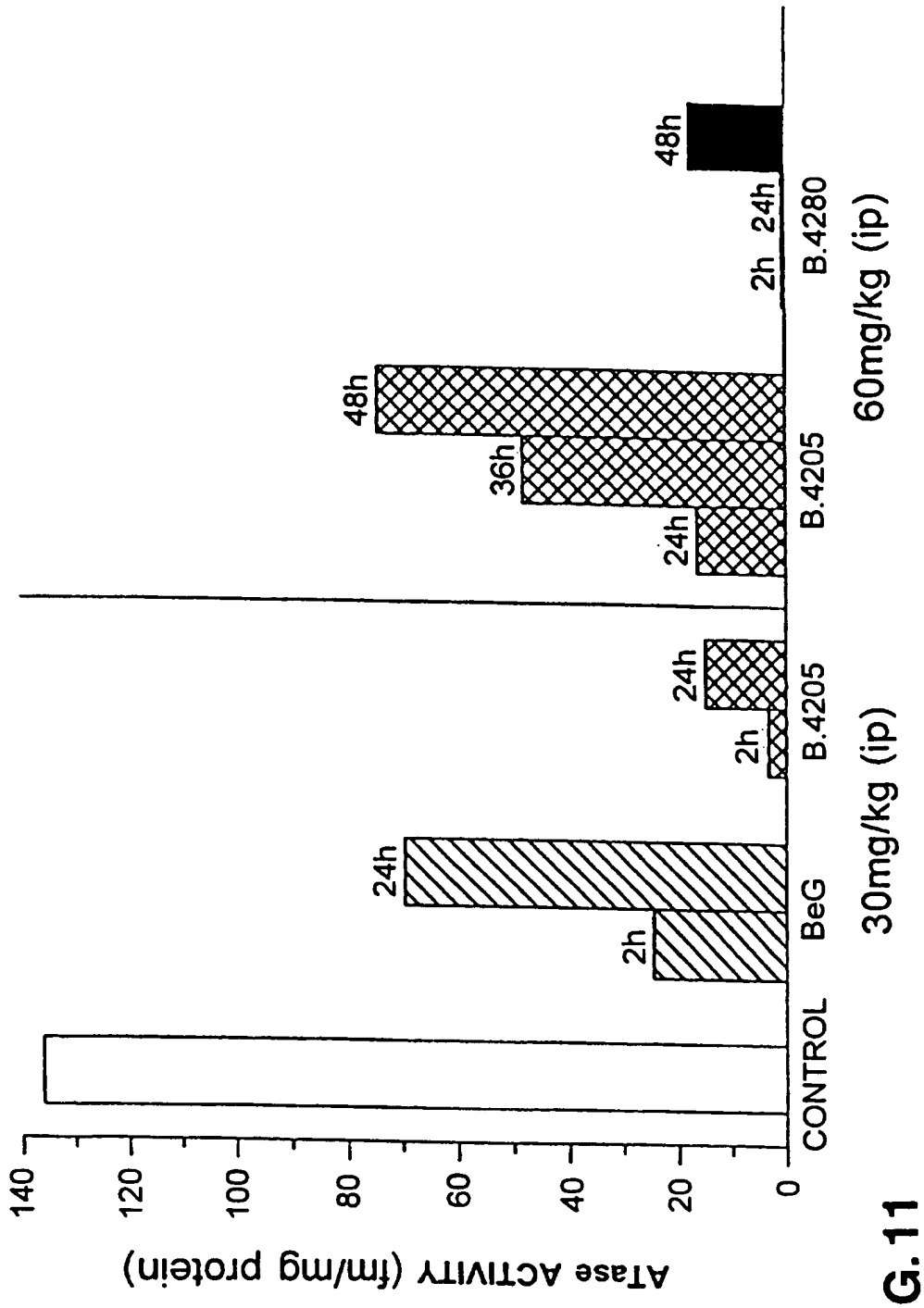
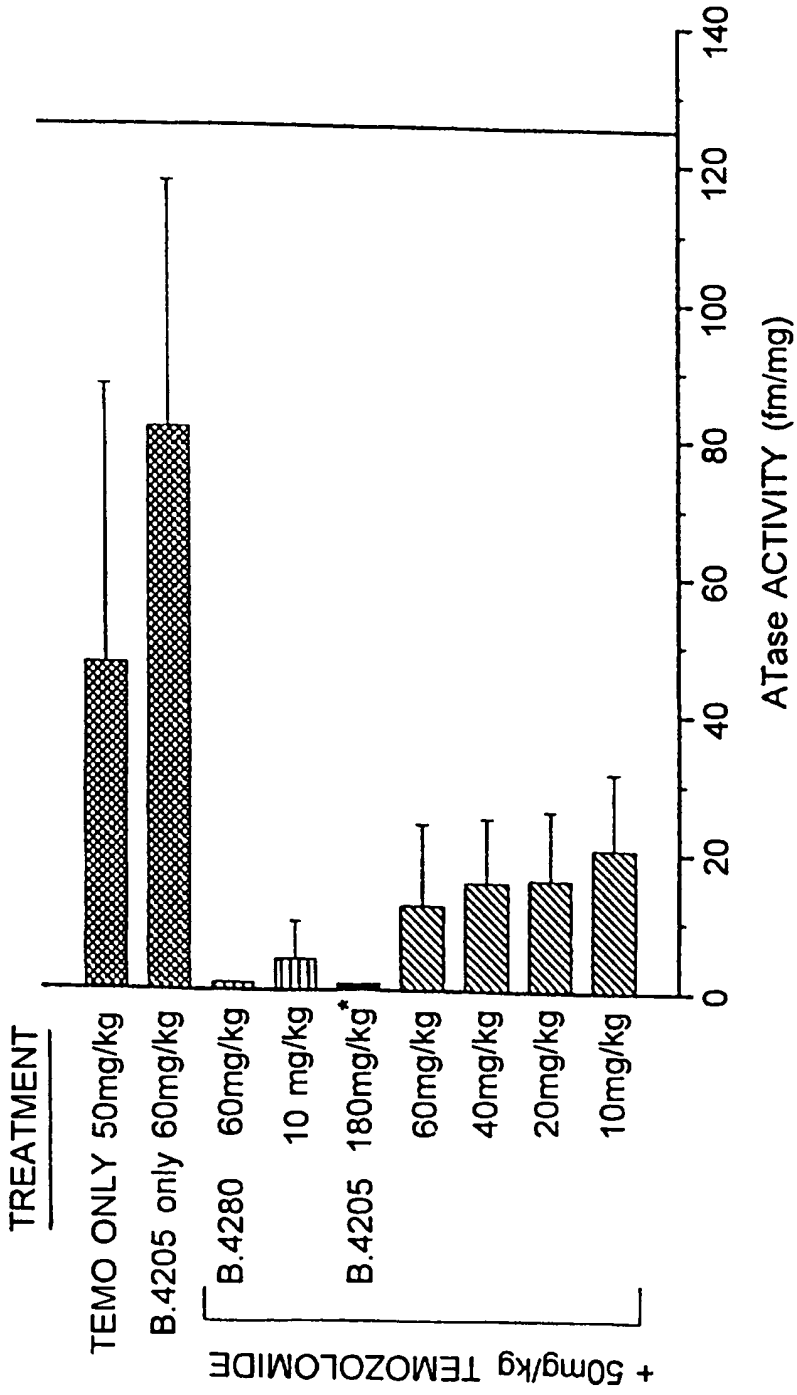

ATase ACTIVITY (fm/mg protein)
140
120
100
80
60
40
20
0
CONTROL
BeG
2h
24h
B.4205
2h
24h
30mg/kg (ip)
B.4205
24h
36h
48h
B.4280
2h
24h
48h
60mg/kg (ip)

FIG. 11



*180 mg/kg B.4205 + 50mg/kg TEMOZOLOMIDE GIVEN ON DAY 1
FOLLOWED BY 50mg/kg TEMOZOLOMIDE ONLY ON DAYS 2 + 3

FIG. 12

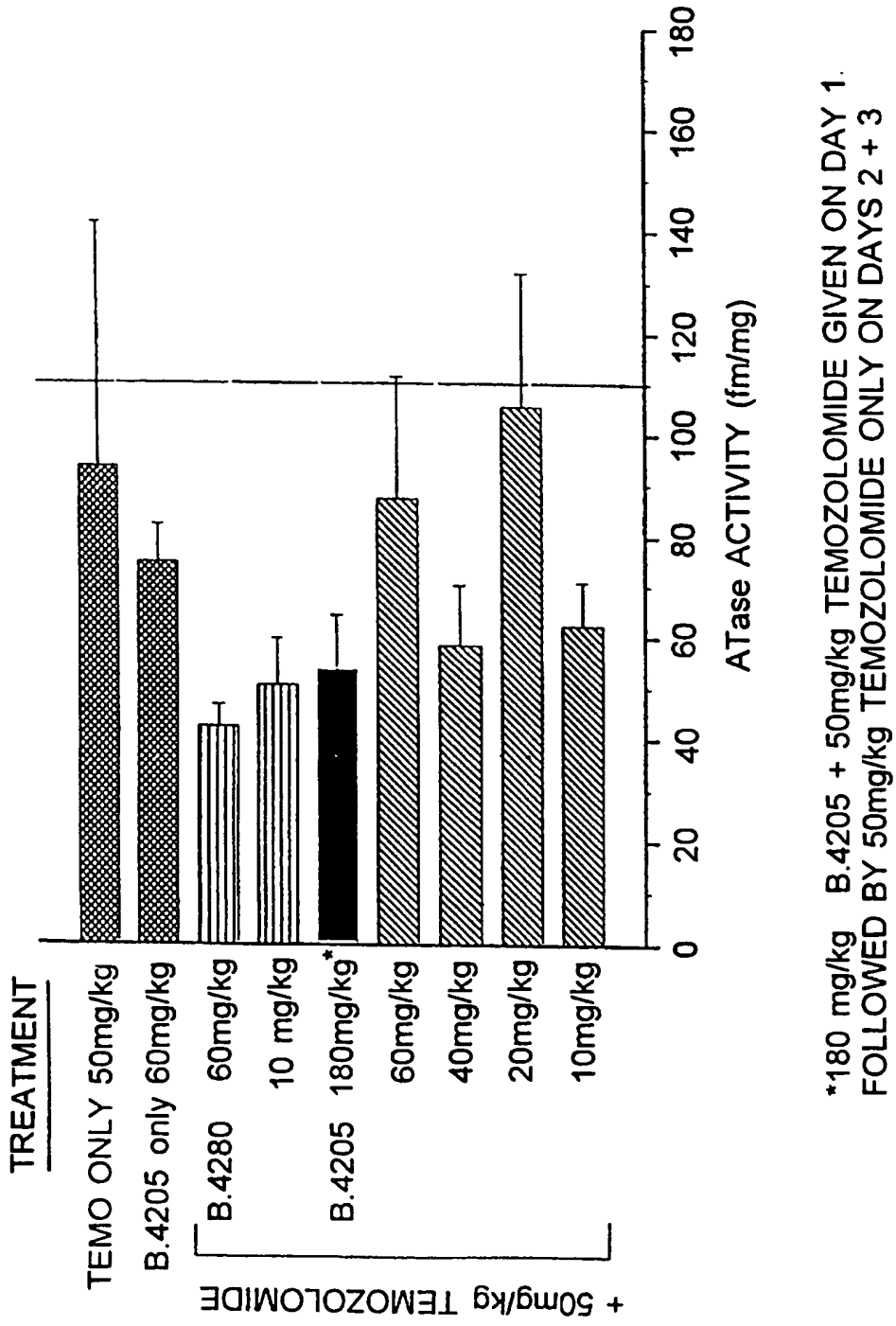

TREATMENT
TEMO ONLY 50mg/kg
B.4205 only 60mg/kg
B.4280 60mg/kg
10 mg/kg
B.4205 180mg/kg*
60mg/kg
40mg/kg
20mg/kg
10mg/kg
+ 50mg/kg TEMOZOLOMIDE
0
20
40
60
80
100
120
140
160
180
ATase ACTIVITY (fm/mg)
*180 mg/kg B.4205 + 50mg/kg TEMOZOLOMIDE GIVEN ON DAY 1.
FOLLOWED BY 50mg/kg TEMOZOLOMIDE ONLY ON DAYS 2 + 3

FIG. 13

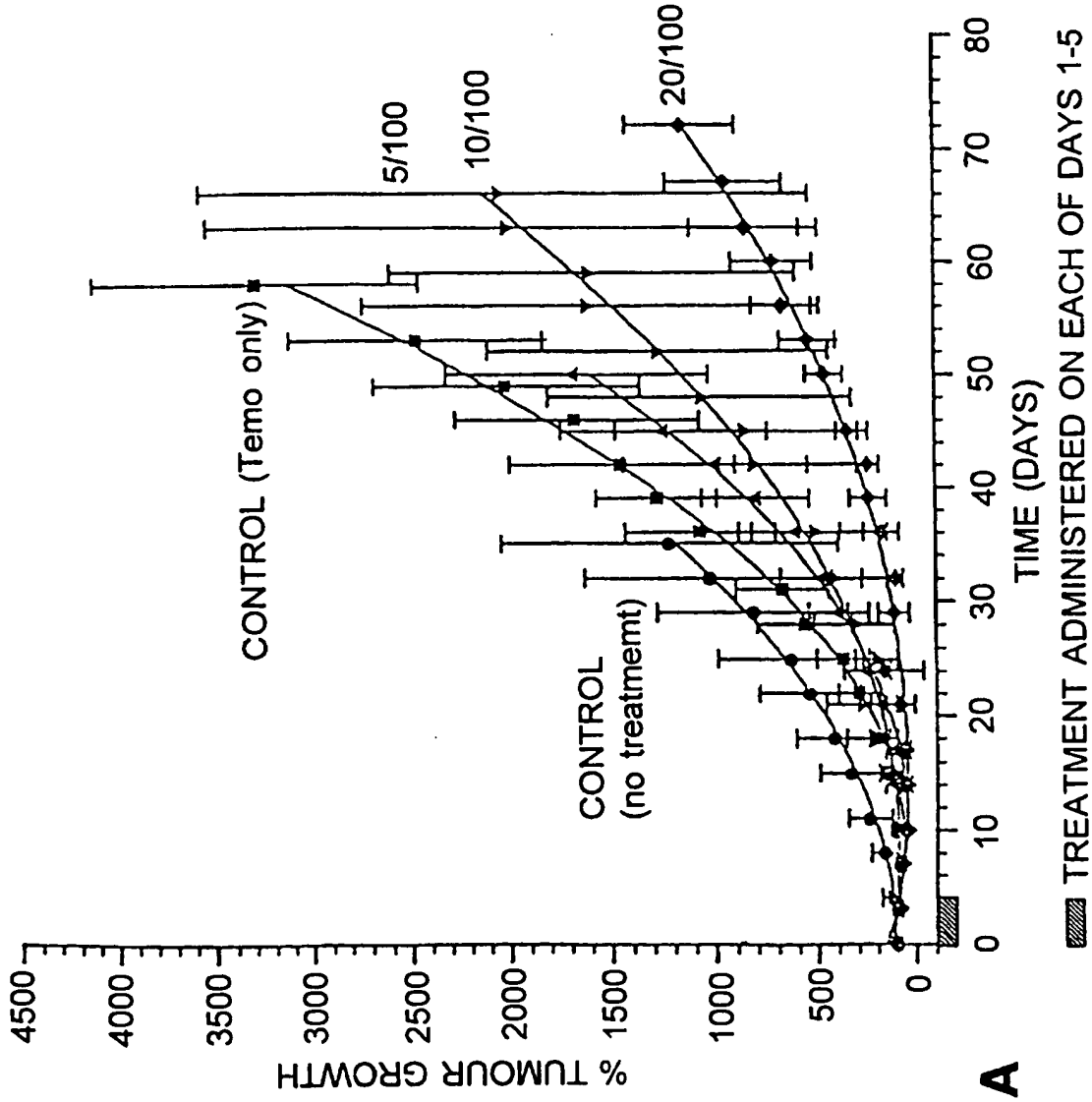

% TUMOUR GROWTH
4500
4000
3500
3000
2500
2000
1500
1000
500
0
0
10
20
30
40
50
60
70
80
TIME (DAYS)
TREATMENT ADMINISTERED ON EACH OF DAYS 1-5
CONTROL (Temo only)
CONTROL (no treatment)
5/100
10/100
20/100

FIG. 14 A

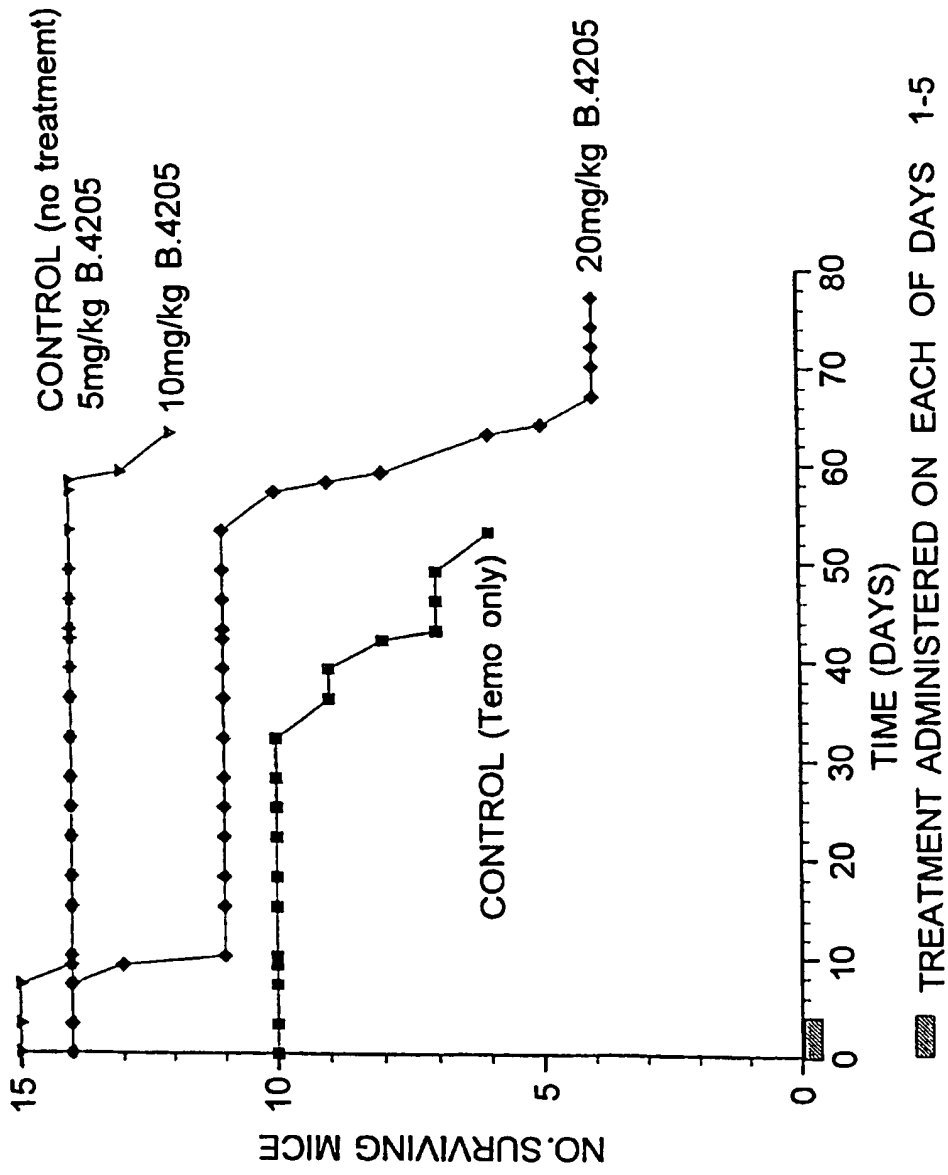
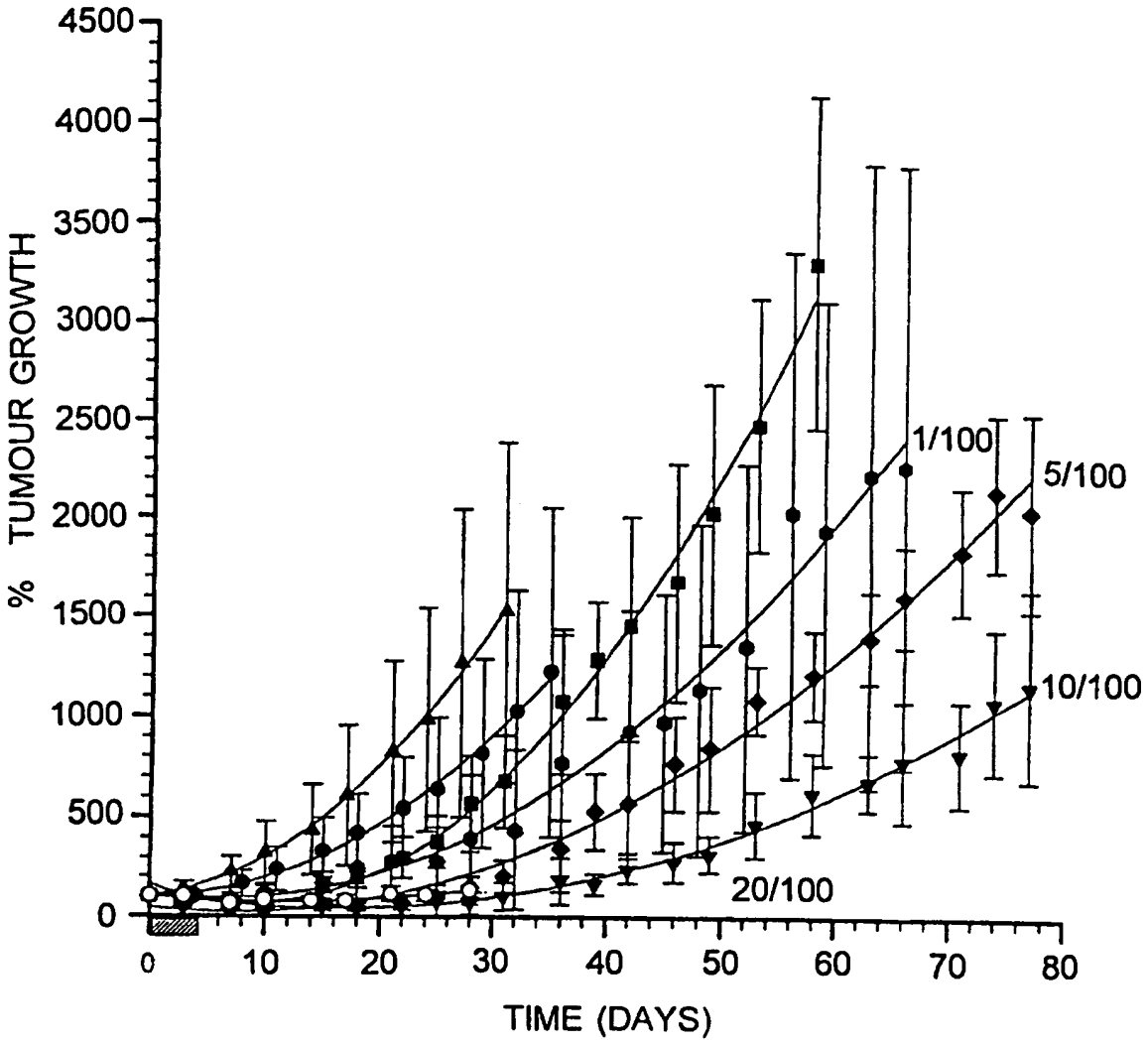

CONTROL (no treatmemt)
5mg/kg B.4205
10mg/kg B.4205
20mg/kg B.4205
CONTROL (Temo only)
NO. SURVIVING MICE
0
5
10
15
0
10
20
30
40
50
60
70
80
TIME (DAYS)
TREATMENT ADMINISTERED ON EACH OF DAYS 1-5

FIG. 14B



TREATMENT ADMINISTERED ON THESE DAYS

▲ CONTROL (20mg/kg B4280)

● CONTROL (no treatmemt)

■ CONTROL (Temo only)

FIG. 15A

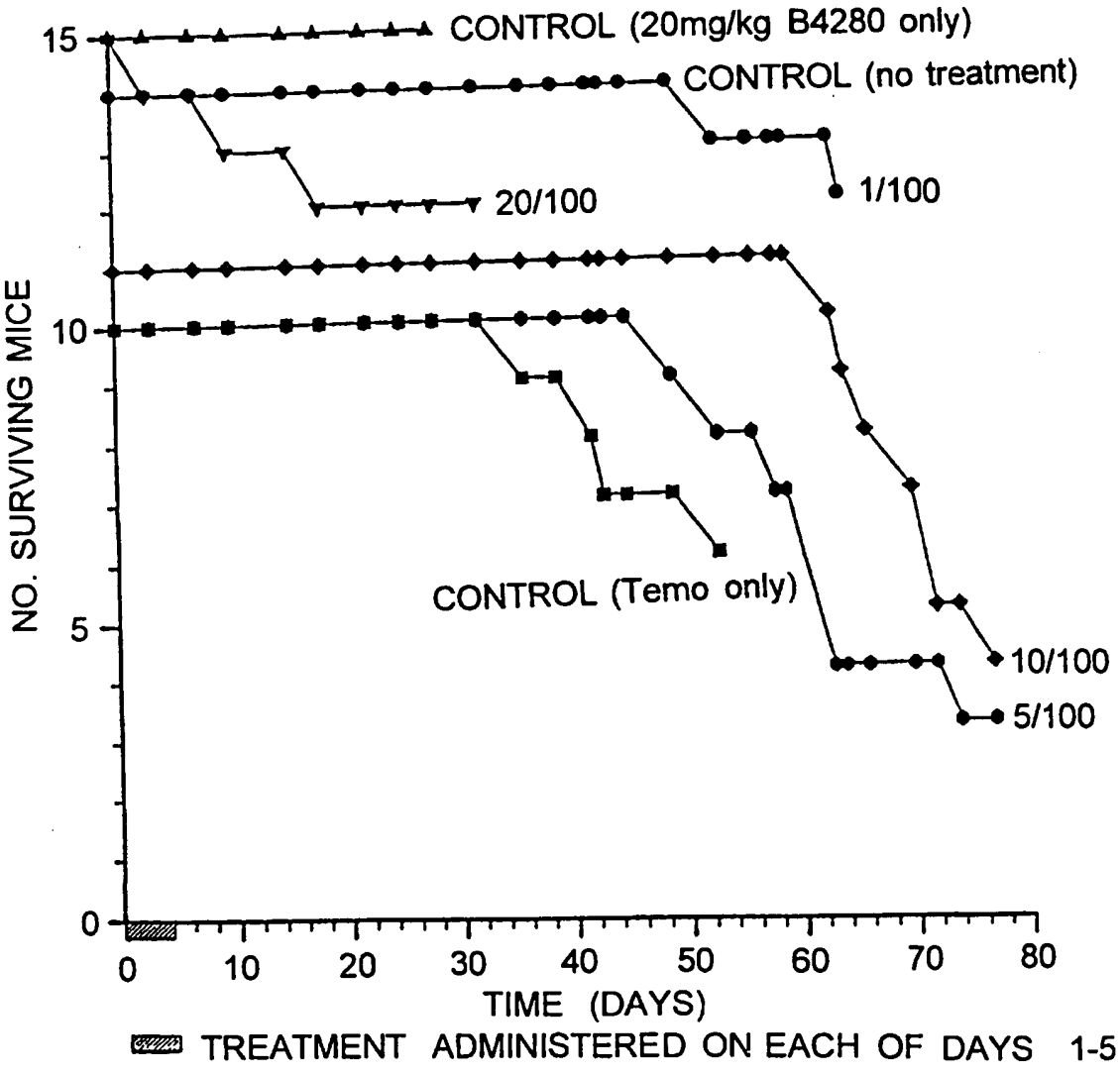

CONTROL (20mg/kg B4280 only)
CONTROL (no treatment)
1/100
20/100
CONTROL (Temo only)
10/100
5/100
NO. SURVIVING MICE
15
10
5
0
0
10
20
30
40
50
60
70
80
TIME (DAYS)
TREATMENT ADMINISTERED ON EACH OF DAYS 1-5

FIG. 15B

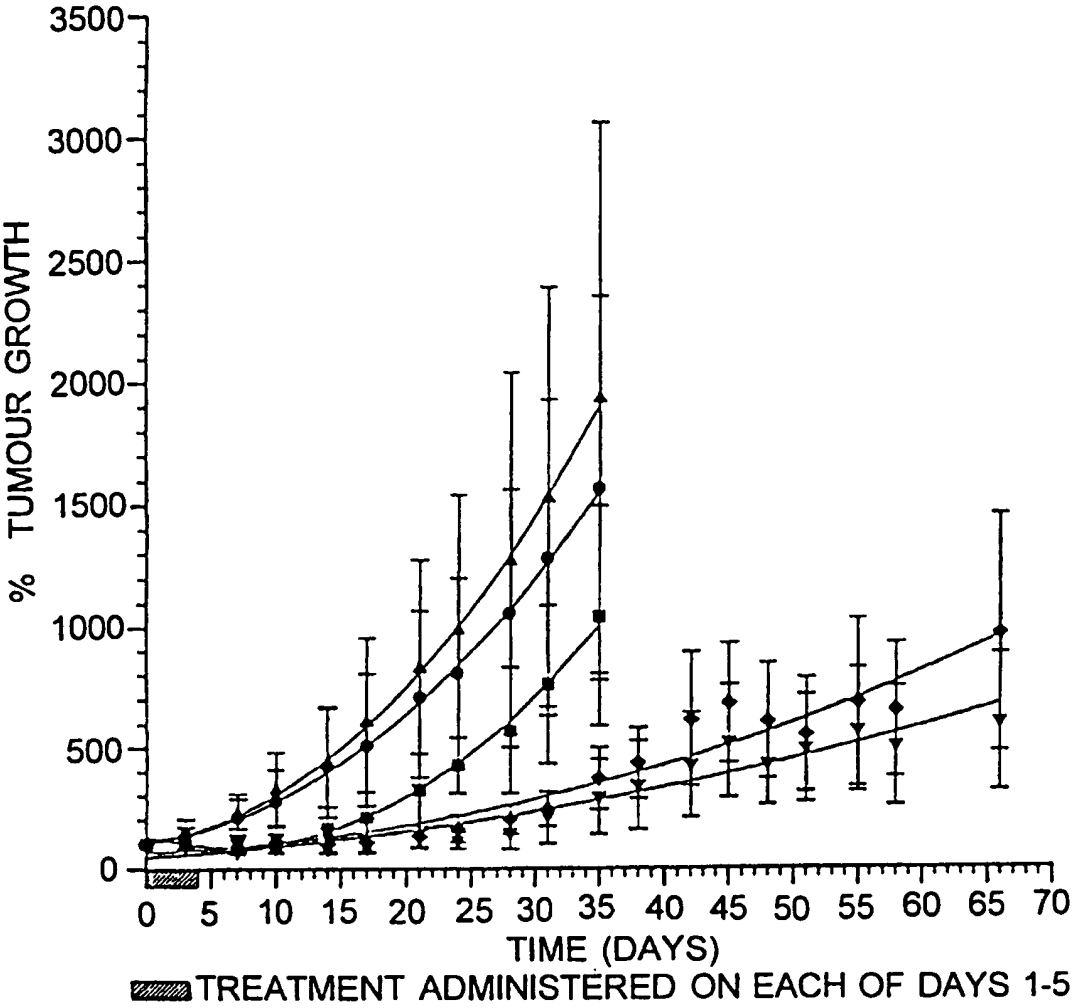

3500
3000
2500
2000
1500
1000
500
0
% TUMOUR GROWTH
0 5 10 15 20 25 30 35 40 45 50 55 60 65 70
TIME (DAYS)
TREATMENT ADMINISTERED ON EACH OF DAYS 1-5
▲ CONTROL (20mg/kg B. 4280 only)
● CONTROL (no treatmemt)
■ CONTROL (100mg/kg Temo only)
◆ 30mg/kg B.4280 + 100mg/kg TEMO (po)
▼ 20mg/kg B.4280 + 100mg/kg TEMO (ip)

FIG. 16 A

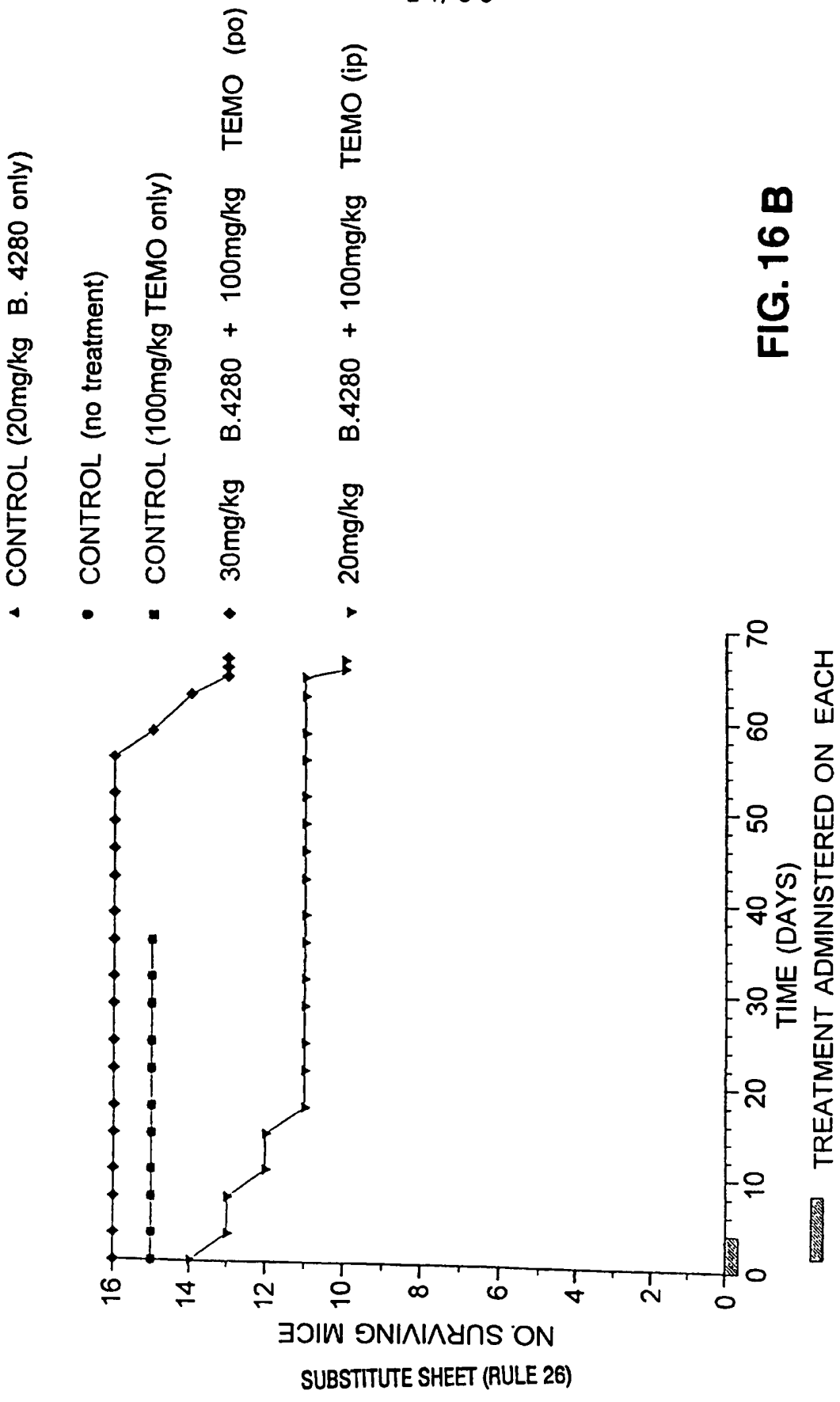

▲ CONTROL (20mg/kg B. 4280 only)
● CONTROL (no treatment)
■ CONTROL (100mg/kg TEMO only)
◆ 30mg/kg B.4280 + 100mg/kg TEMO (po)
▼ 20mg/kg B.4280 + 100mg/kg TEMO (ip)
NO. SURVIVING MICE
0
2
4
6
8
10
12
14
16
0
10
20
30
40
50
60
70
TIME (DAYS)
TREATMENT ADMINISTERED ON EACH OF DAYS 1-5

FIG. 16 B

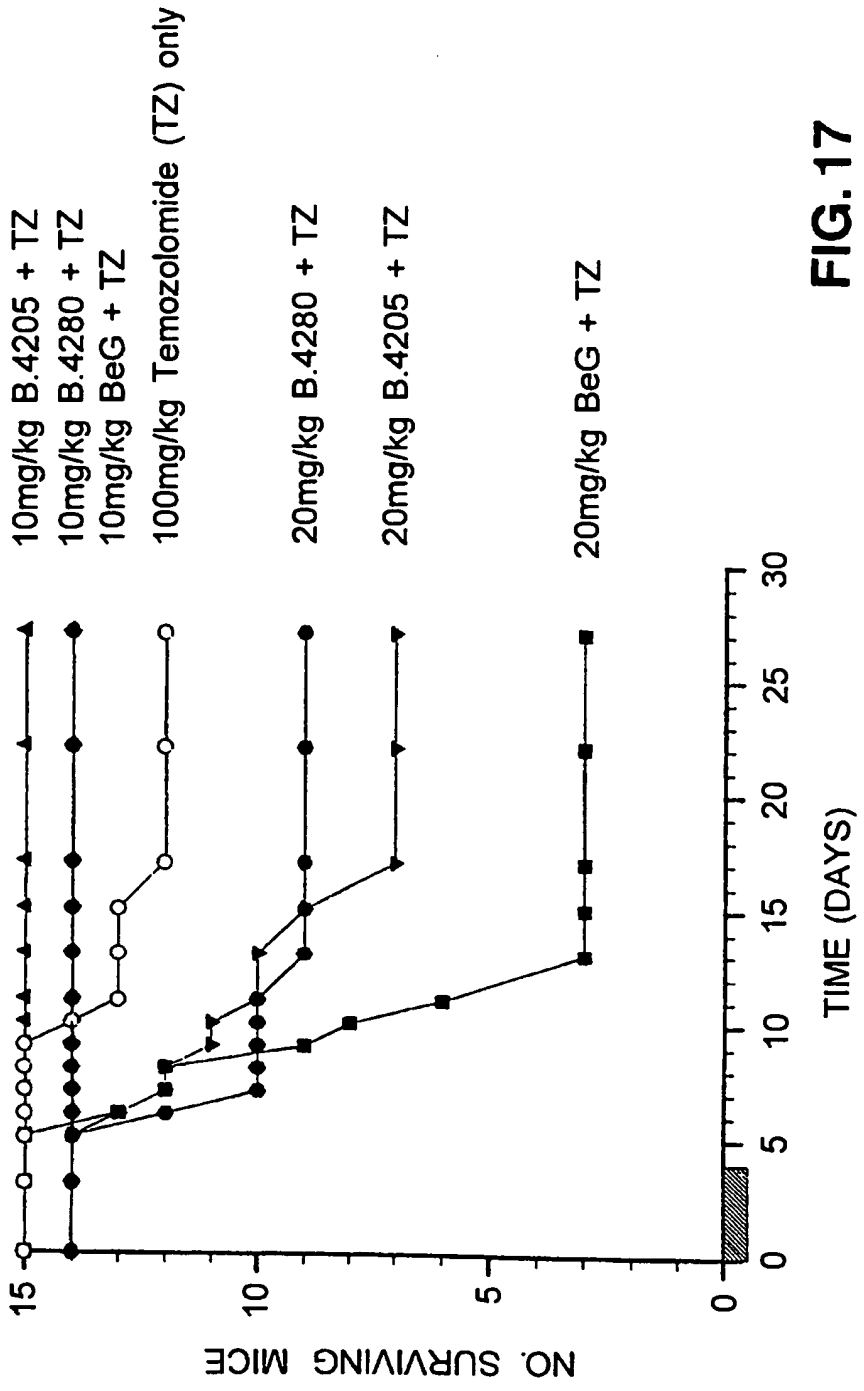
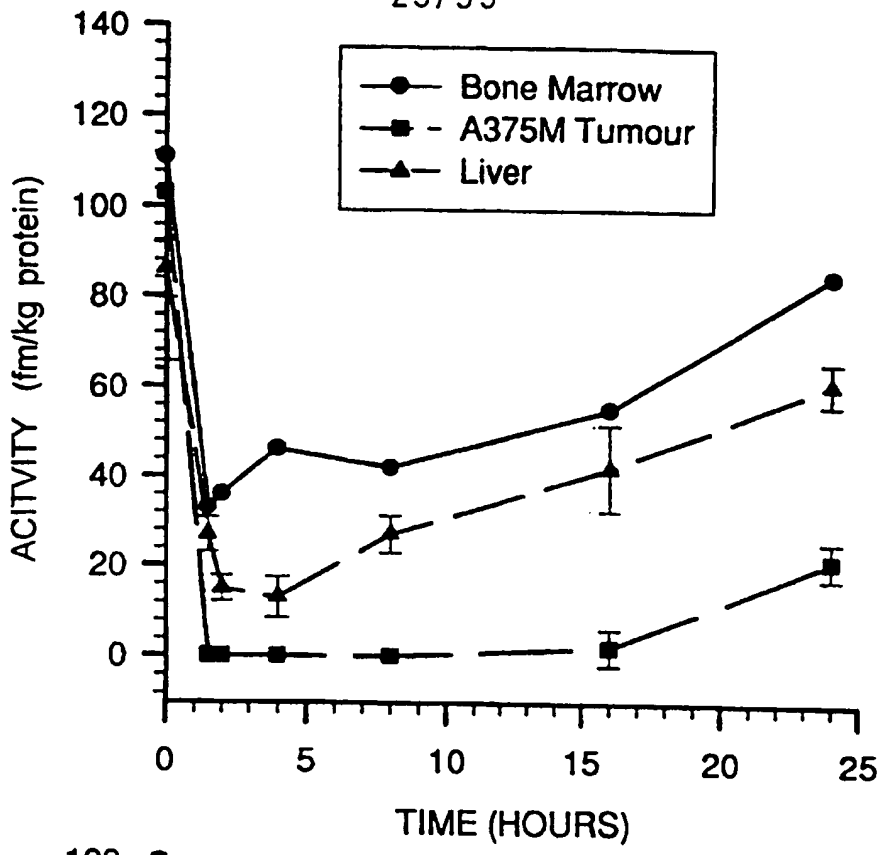

10mg/kg B.4205 + TZ
10mg/kg B.4280 + TZ
10mg/kg BeG + TZ
100mg/kg Temozolomide (TZ) only
20mg/kg B.4280 + TZ
20mg/kg B.4205 + TZ
20mg/kg BeG + TZ
NO. SURVIVING MICE
TIME (DAYS)
0
5
10
15
0
5
10
15
20
25
30

FIG. 17


Bone Marrow
A375M Tumour
Liver
ACITVITY (fm/kg protein)
140
120
100
80
60
40
20
0
0
5
10
15
20
25
TIME (HOURS)

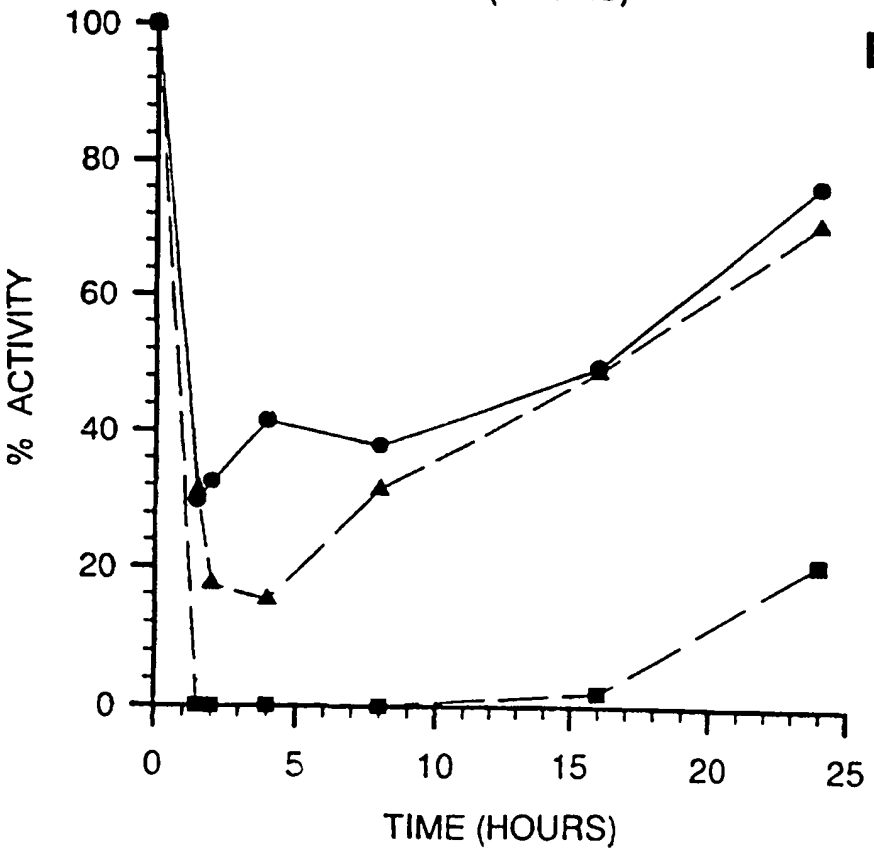
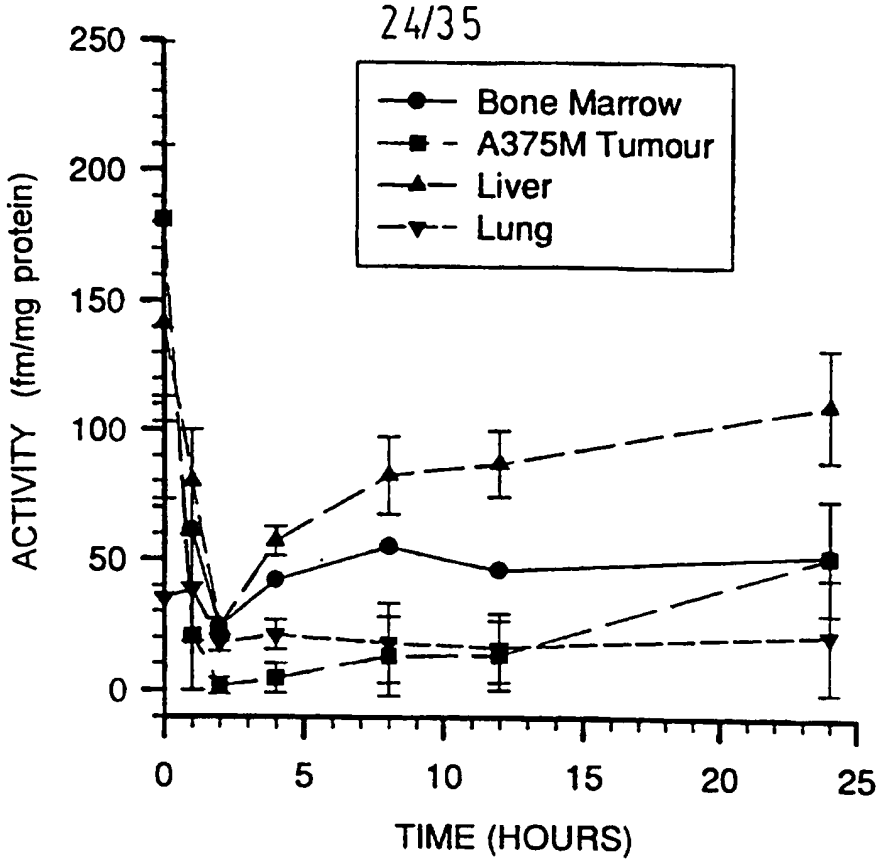

% ACTIVITY
100
80
60
40
20
0
0
5
10
15
20
25
TIME (HOURS)

FIG. 18


Bone Marrow
A375M Tumour
Liver
Lung
ACTIVITY (fm/mg protein)
250
200
150
100
50
0
0
5
10
15
20
25
TIME (HOURS)

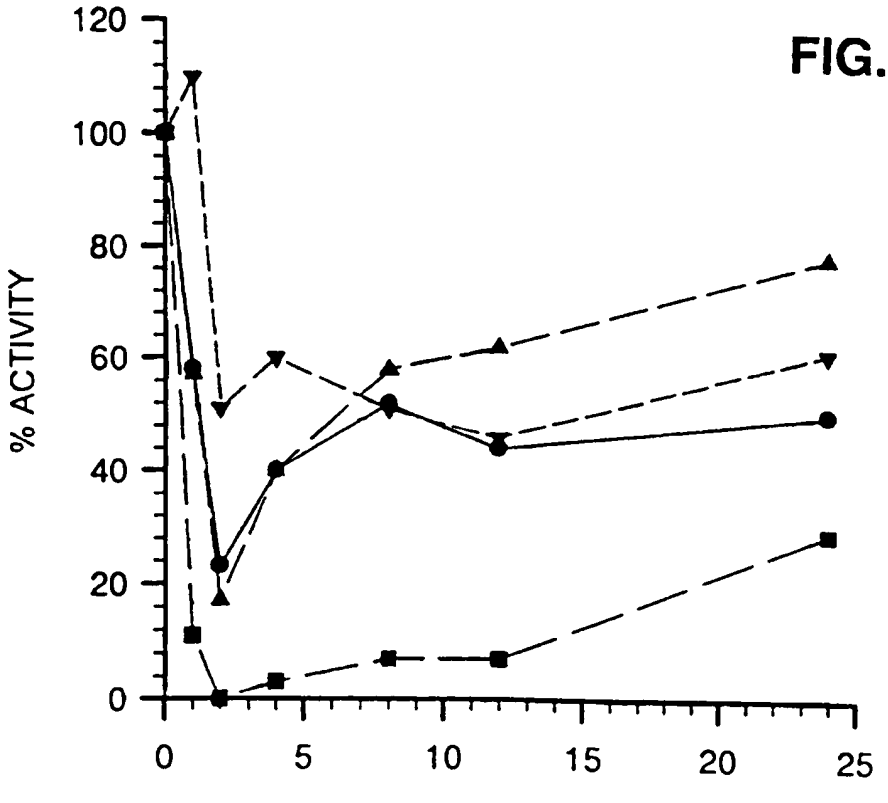

% ACTIVITY
120
100
80
60
40
20
0
0
5
10
15
20
25
TIME (HOURS)

FIG. 19

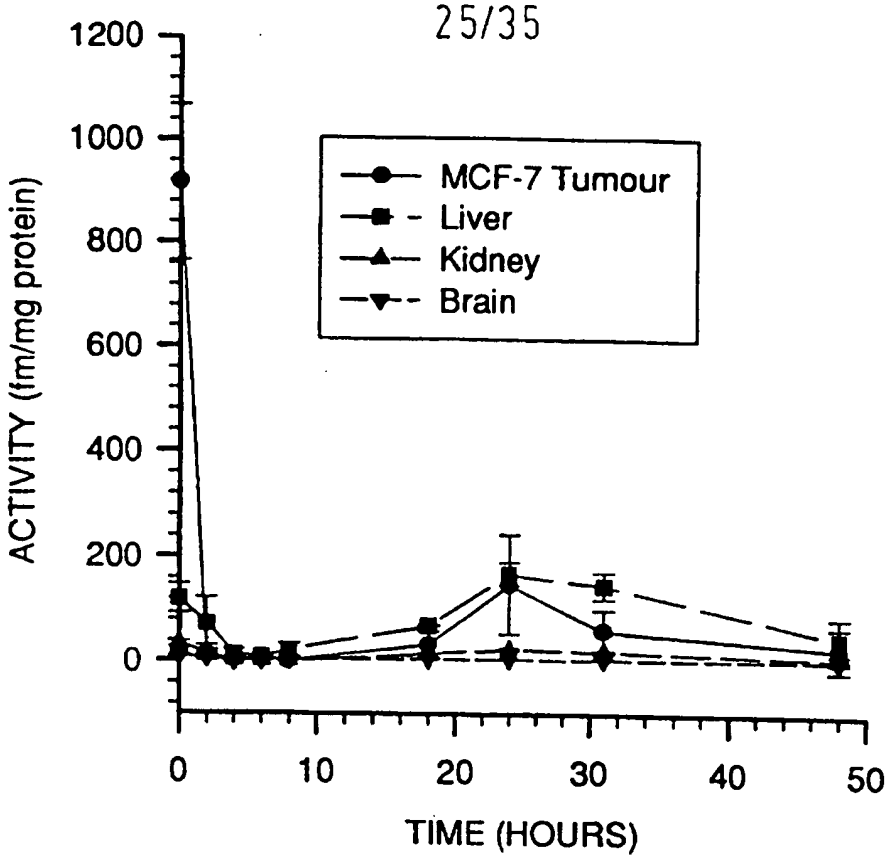
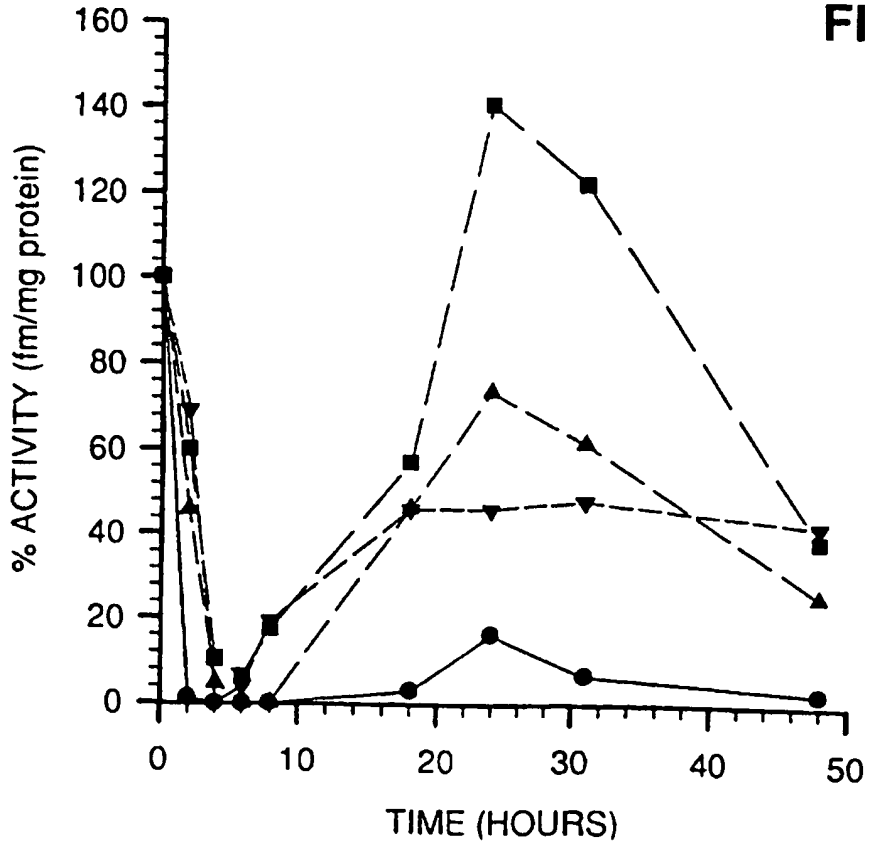

ACTIVITY (fm/mg protein)
1200
1000
800
600
400
200
0
MCF-7 Tumour
Liver
Kidney
Brain
0
10
20
30
40
50
TIME (HOURS)

FIG. 20


% ACTIVITY (fm/mg protein)
160
140
120
100
80
60
40
20
0
0
10
20
30
40
50
TIME (HOURS)

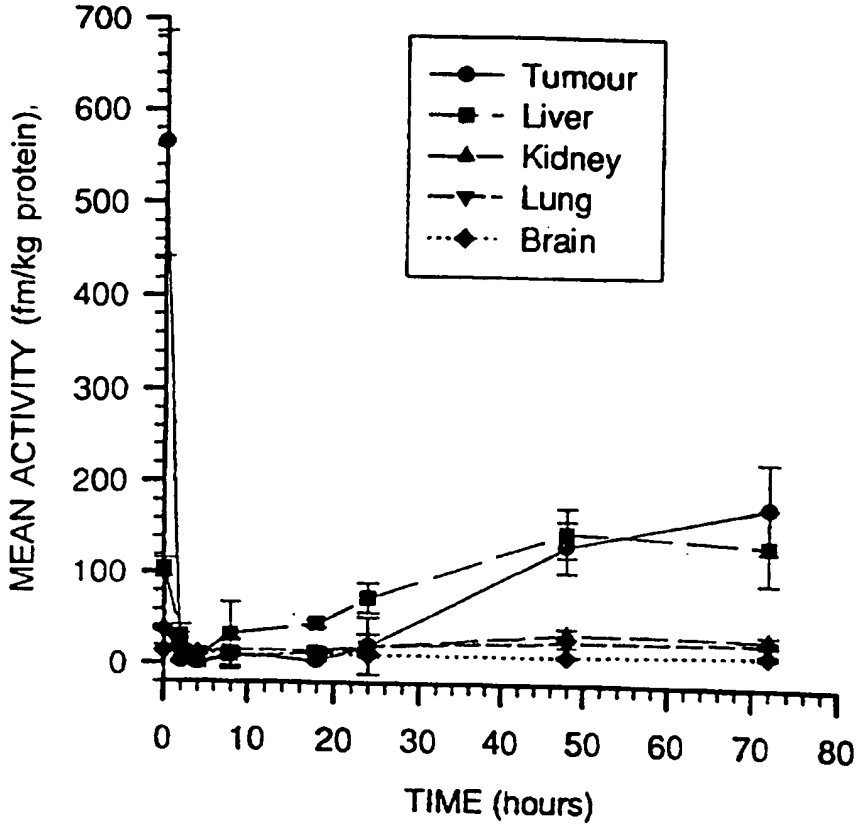
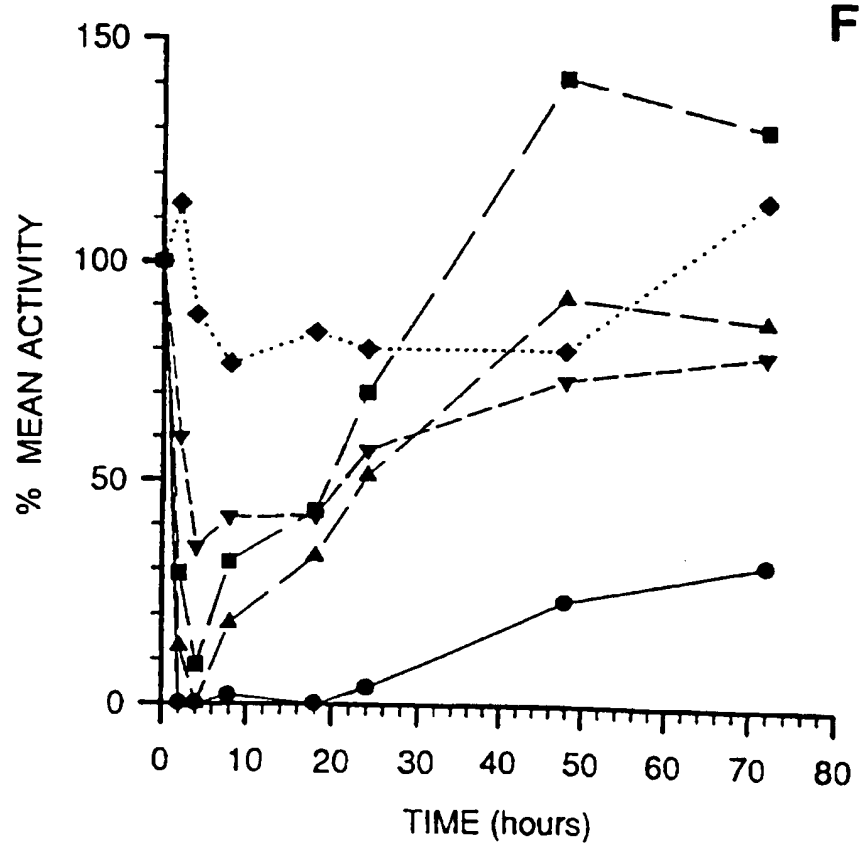

MEAN ACTIVITY (fm/kg protein)
700
600
500
400
300
200
100
0
Tumour
Liver
Kidney
Lung
Brain
0
10
20
30
40
50
60
70
80
TIME (hours)

FIG. 21


% MEAN ACTIVITY
150
100
50
0
0
10
20
30
40
50
60
70
80
TIME (hours)

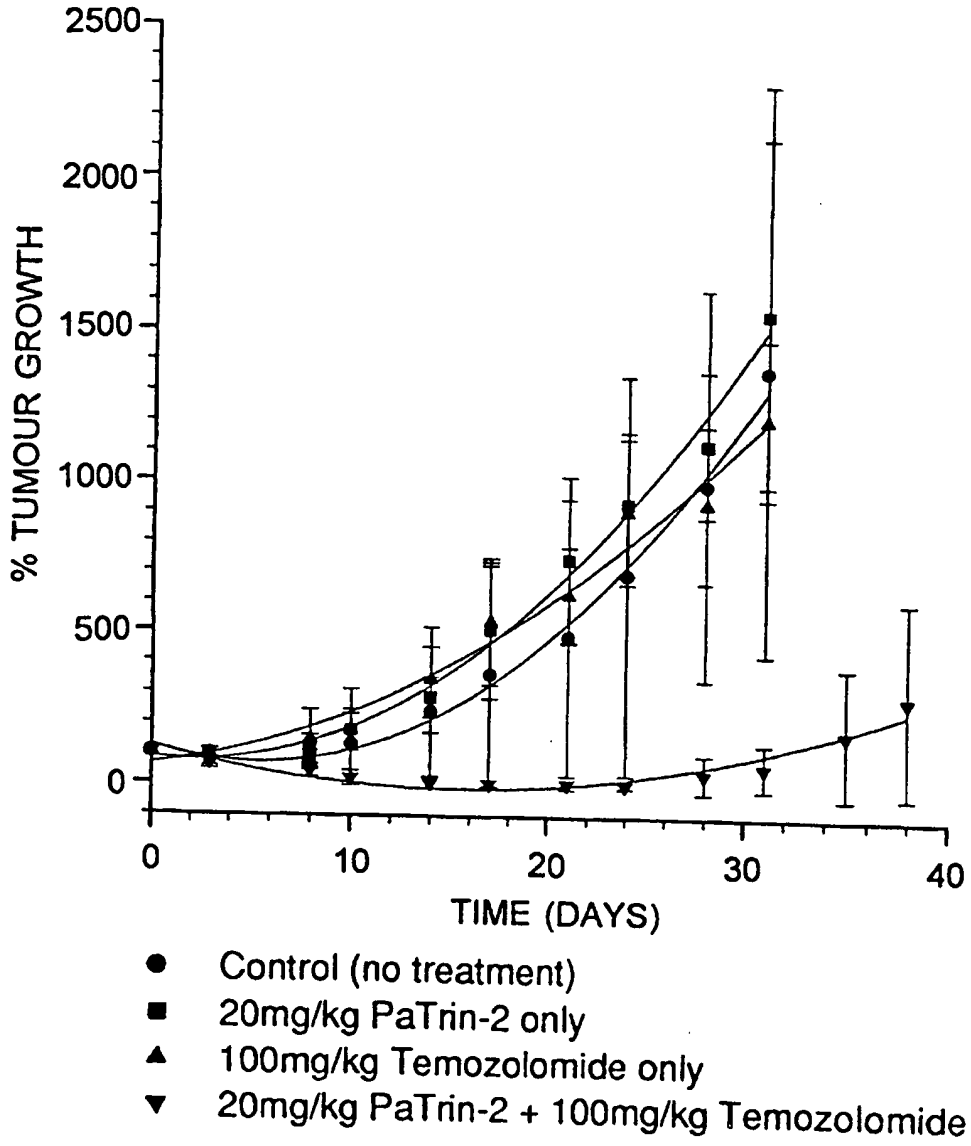

2500
2000
1500
1000
500
0
% TUMOUR GROWTH
0
10
20
30
40
TIME (DAYS)
Control (no treatment)
20mg/kg PaTrin-2 only
100mg/kg Temozolomide only
20mg/kg PaTrin-2 + 100mg/kg Temozolomide

FIG. 22

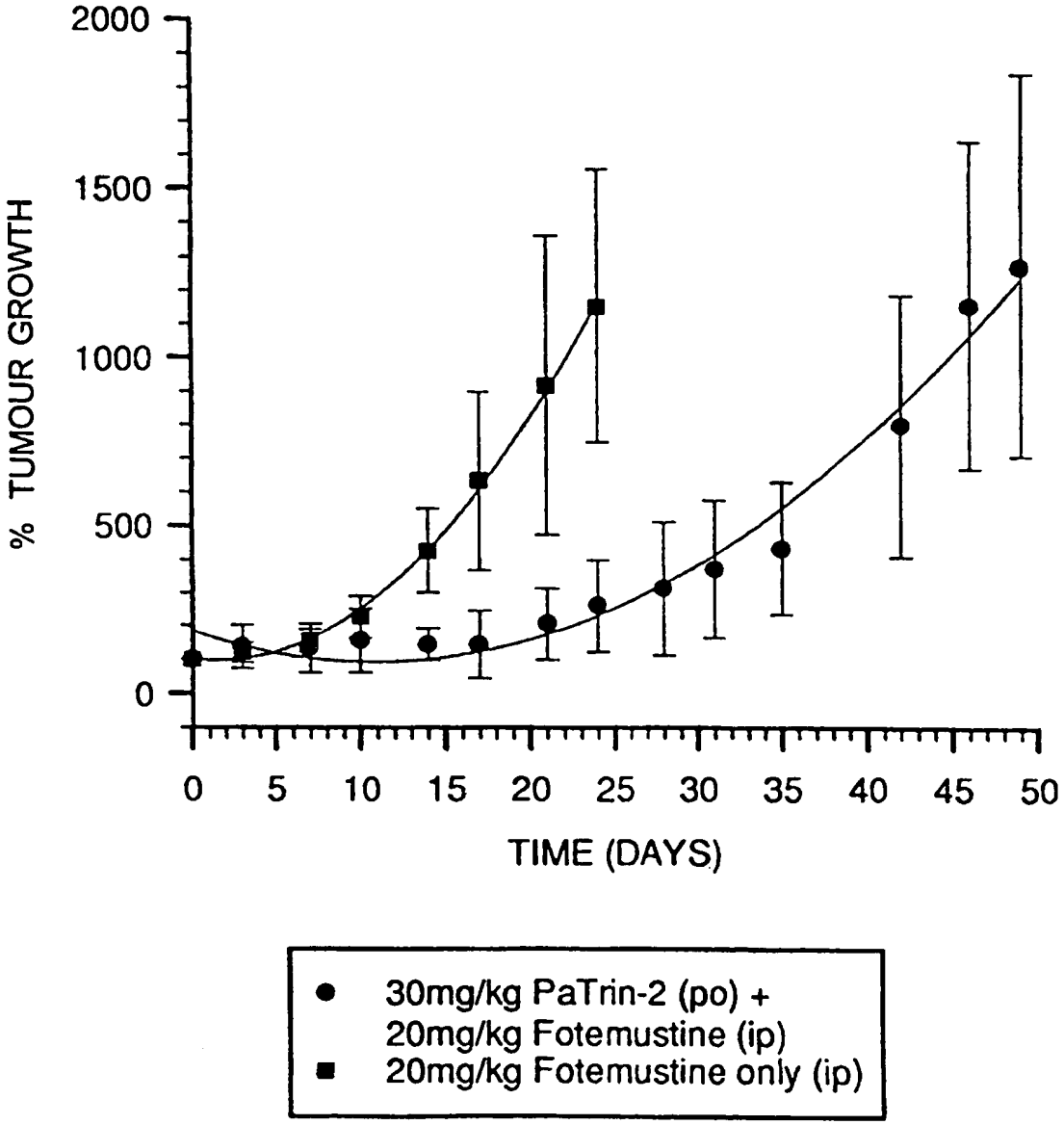

2000
1500
1000
500
0
% TUMOUR GROWTH
0
5
10
15
20
25
30
35
40
45
50
TIME (DAYS)
30mg/kg PaTrin-2 (po) +
20mg/kg Fotemustine (ip)
20mg/kg Fotemustine only (ip)

FIG. 23 A

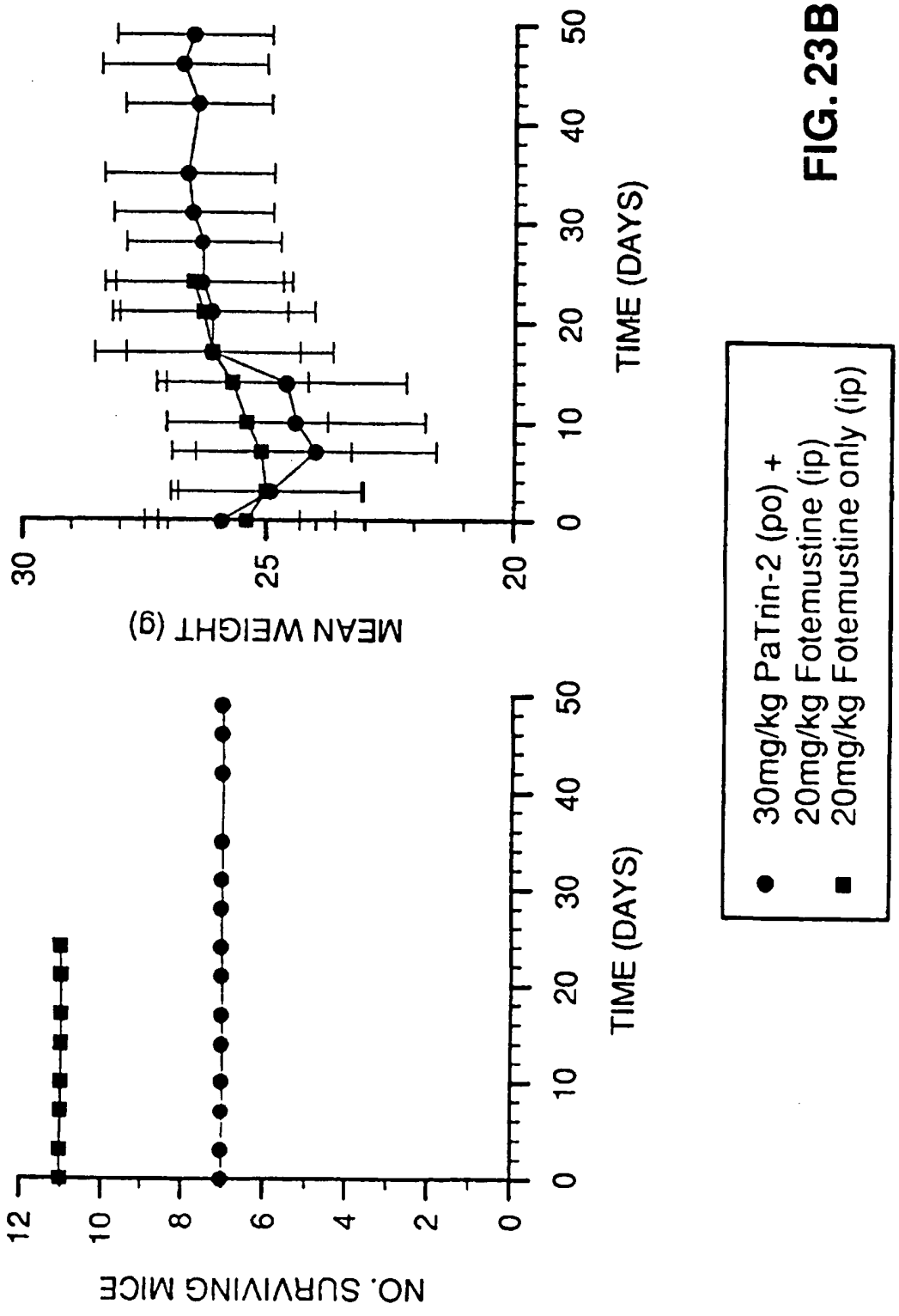

MEAN WEIGHT (g)
30
25
20
0
10
20
30
40
50
TIME (DAYS)
NO. SURVIVING MICE
12
10
8
6
4
2
0
0
10
20
30
40
50
TIME (DAYS)
30mg/kg PaTrin-2 (po) +
20mg/kg Fotemustine (ip)
20mg/kg Fotemustine only (ip)

FIG. 23B

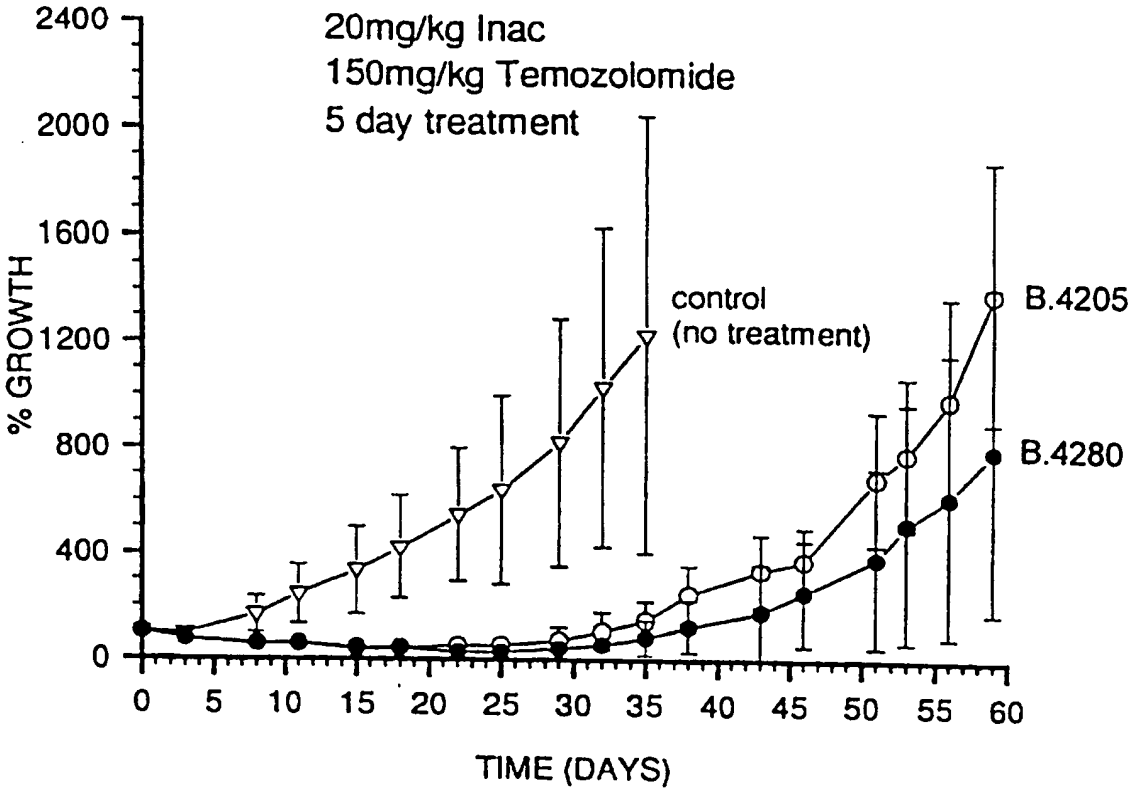
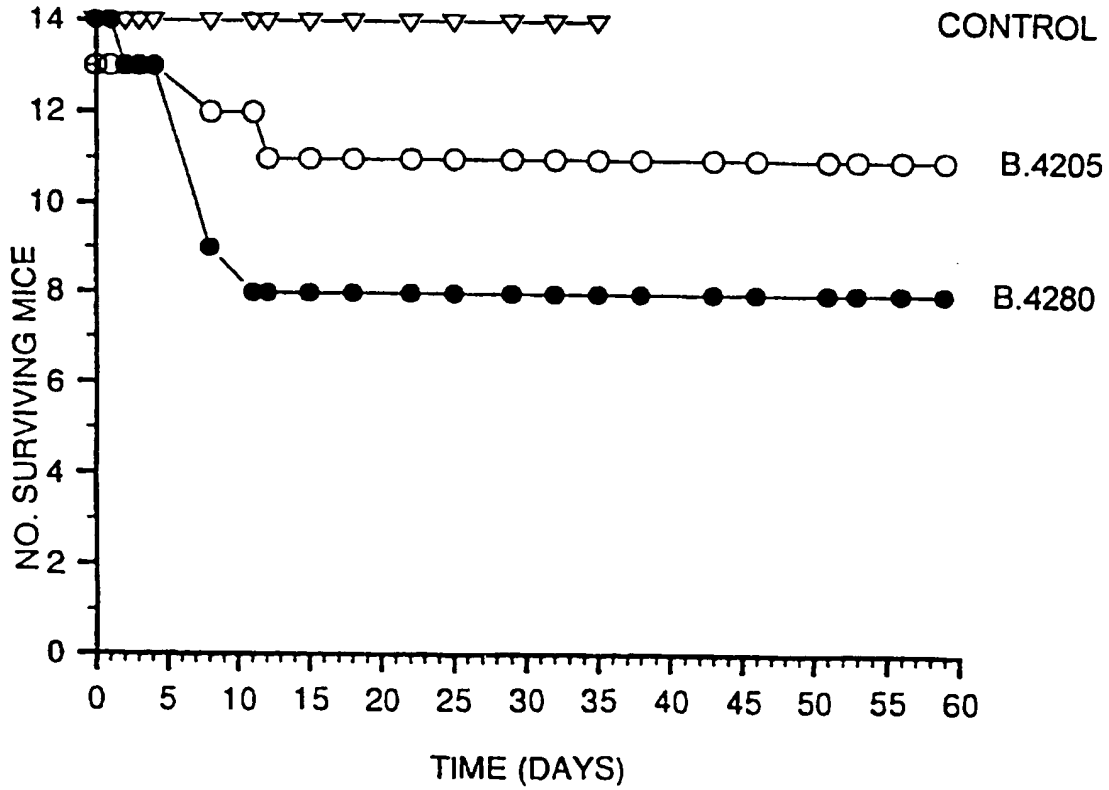
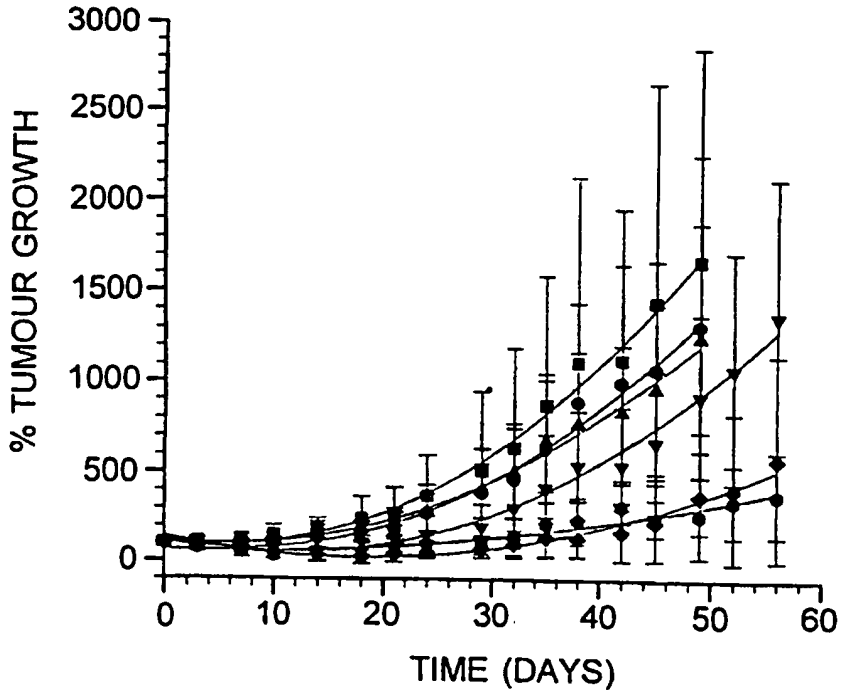
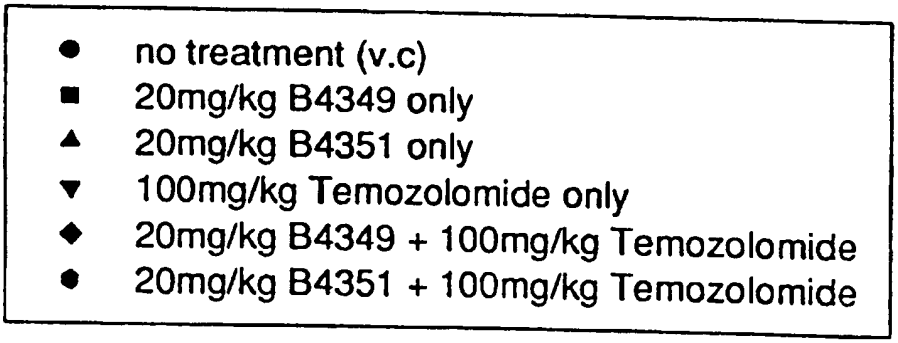
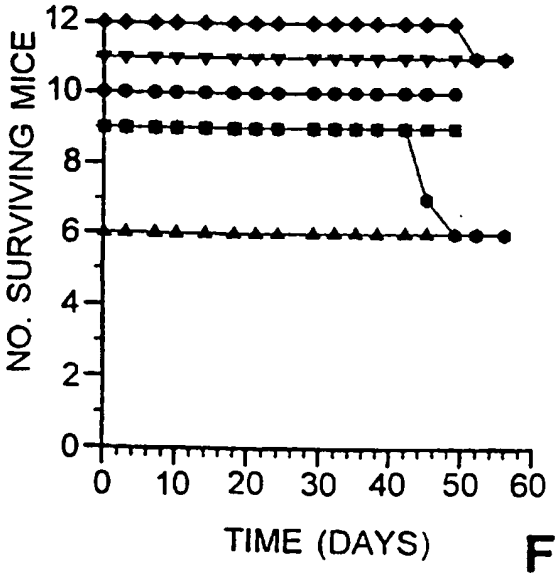

20mg/kg Inac
150mg/kg Temozolomide
5 day treatment
% GROWTH
2400
2000
1600
1200
800
400
0
control
(no treatment)
B.4205
B.4280
0 5 10 15 20 25 30 35 40 45 50 55 60
TIME (DAYS)

FIG. 24


CONTROL
B.4205
B.4280
NO. SURVIVING MICE
14
12
10
8
6
4
2
0
0 5 10 15 20 25 30 35 40 45 50 55 60
TIME (DAYS)


% TUMOUR GROWTH
3000
2500
2000
1500
1000
500
0
0
10
20
30
40
50
60
TIME (DAYS)


no treatment (v.c)
20mg/kg B4349 only
20mg/kg B4351 only
100mg/kg Temozolomide only
20mg/kg B4349 + 100mg/kg Temozolomide
20mg/kg B4351 + 100mg/kg Temozolomide


NO. SURVIVING MICE
12
10
8
6
4
2
0
0
10
20
30
40
50
60
TIME (DAYS)

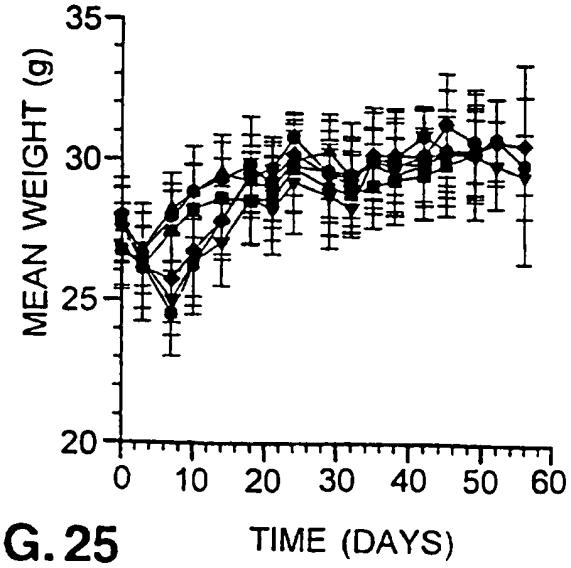

MEAN WEIGHT (g)
35
30
25
20
0
10
20
30
40
50
60
TIME (DAYS)

FIG. 25

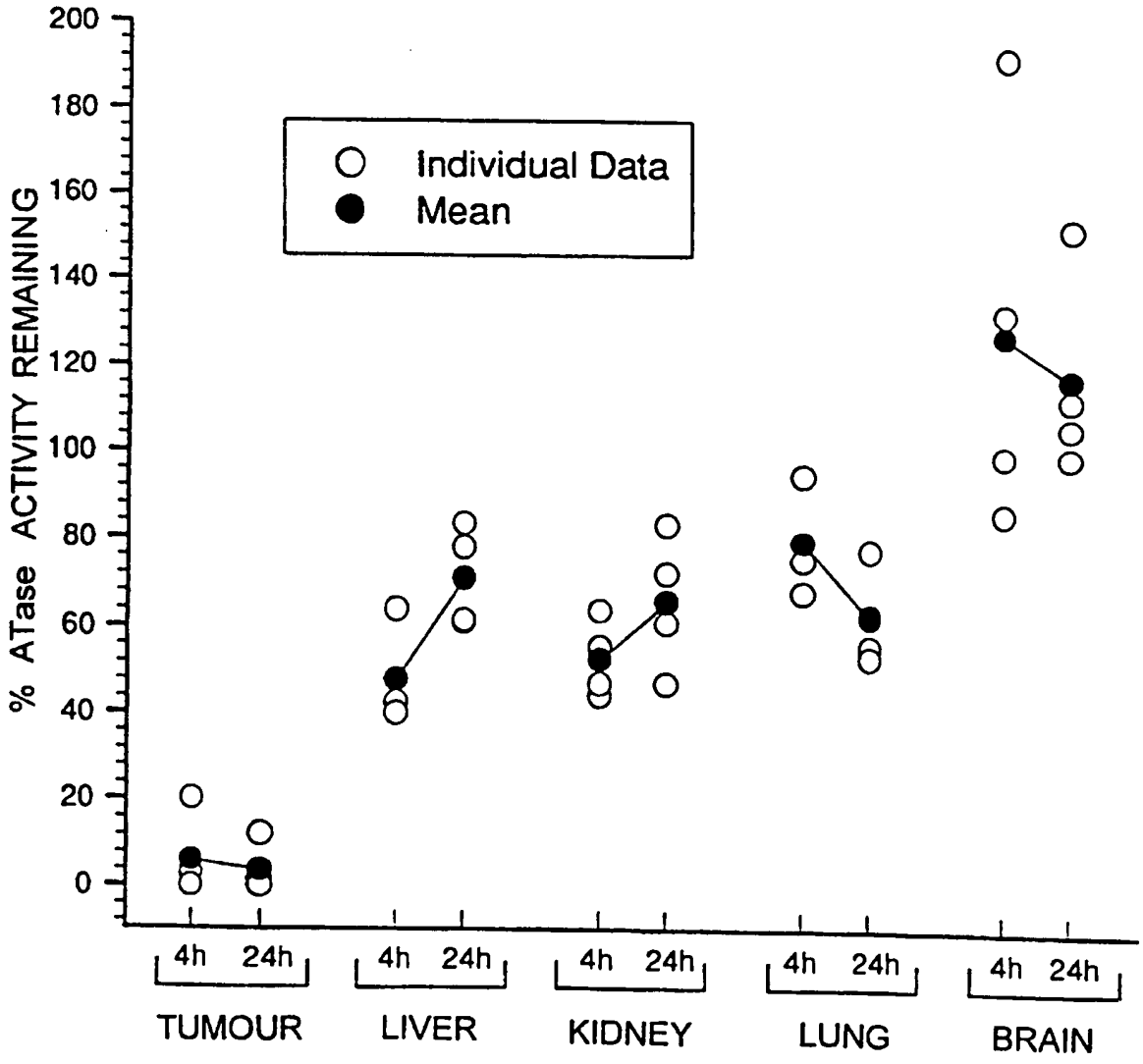

% ATase ACTIVITY REMAINING
200
180
160
140
120
100
80
60
40
20
0
Individual Data
Mean
4h 24h
TUMOUR
LIVER
KIDNEY
LUNG
BRAIN

FIG. 26

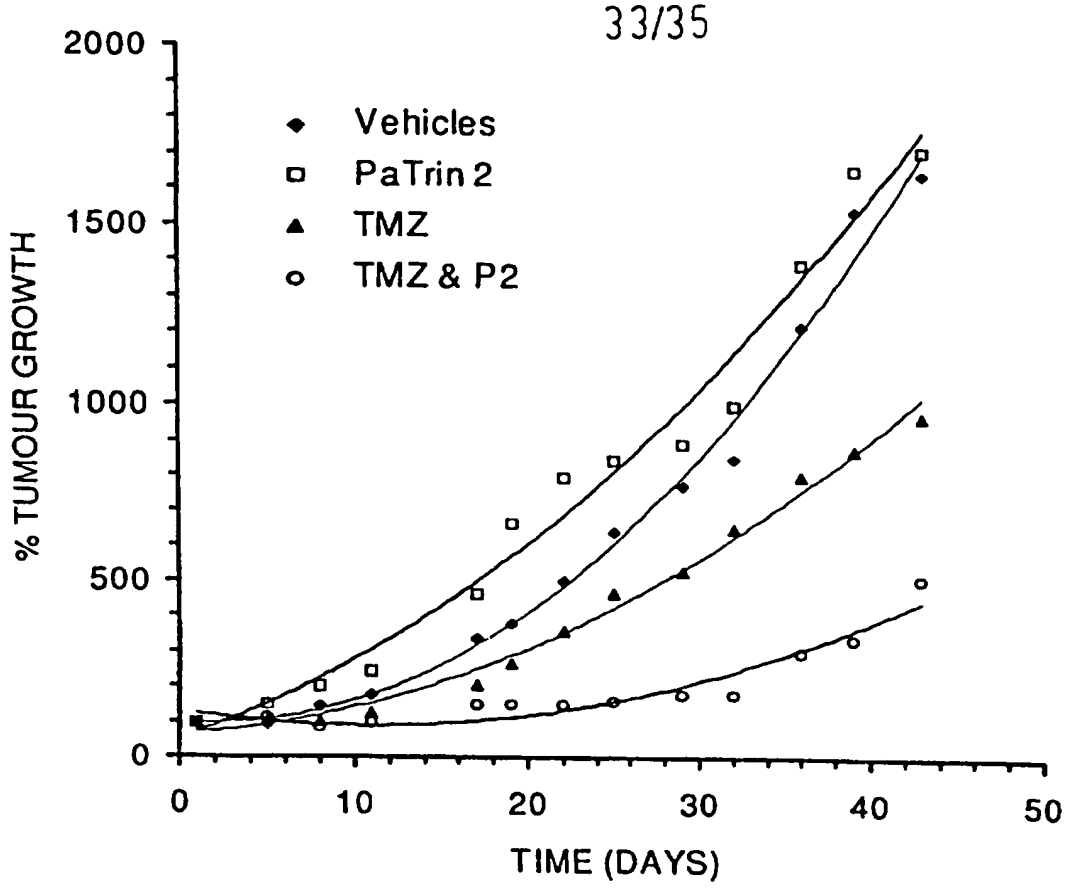
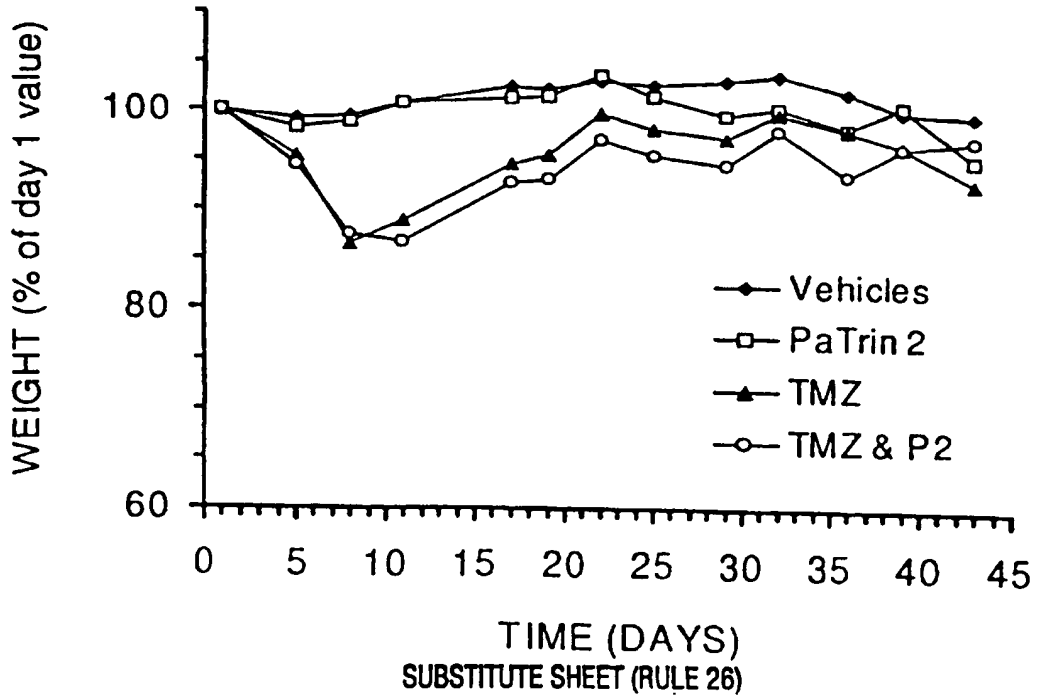

2000
1500
1000
500
0
% TUMOUR GROWTH
Vehicles
PaTrin 2
TMZ
TMZ & P2
0
10
20
30
40
50
TIME (DAYS)

FIG. 27


100
80
60
WEIGHT (% of day 1 value)
Vehicles
PaTrin 2
TMZ
TMZ & P2
0
5
10
15
20
25
30
35
40
45
TIME (DAYS)

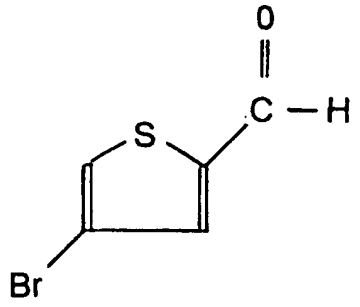
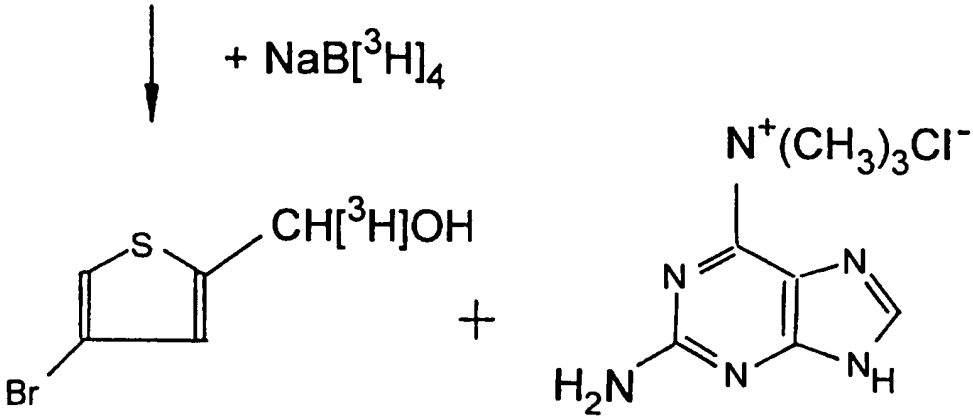
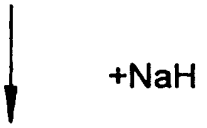


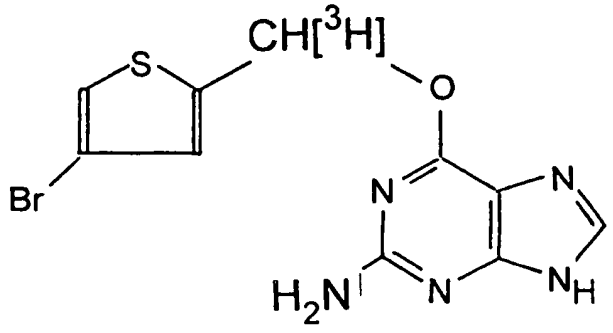
FIG. 28

4-BROMO-2- THIOPHENECARBOXALDEHYDE



[3H]4-BROMOTHENYLALCOHOL GUANINE SALT





[3H]4- BROMOTHENYLGUANINE

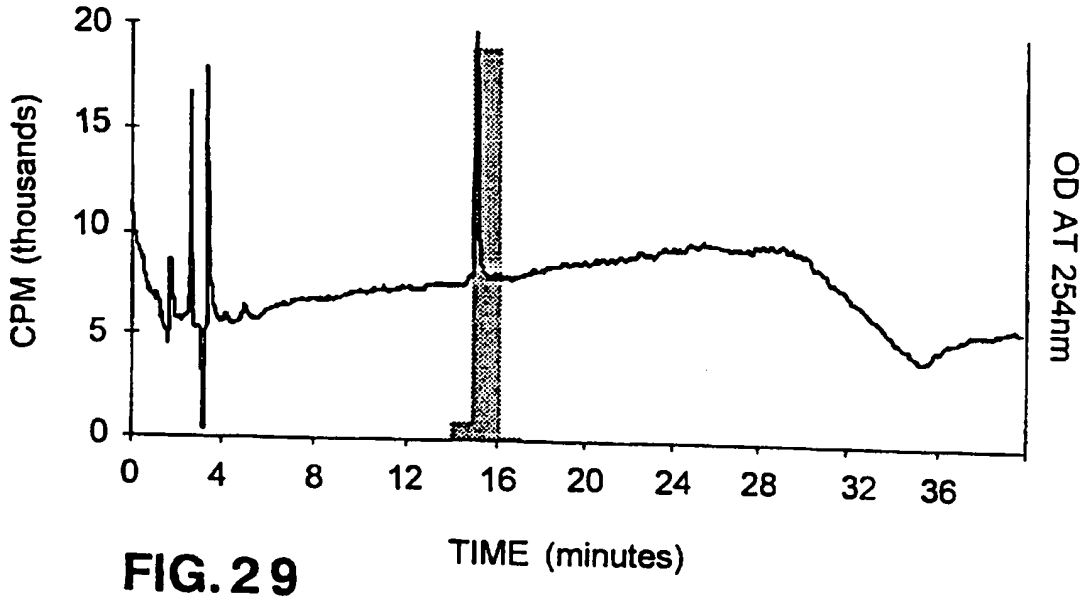

20
15
10
5
0
CPM (thousands)
OD AT 254nm
0
4
8
12
16
20
24
28
32
36
TIME (minutes)

FIG. 29

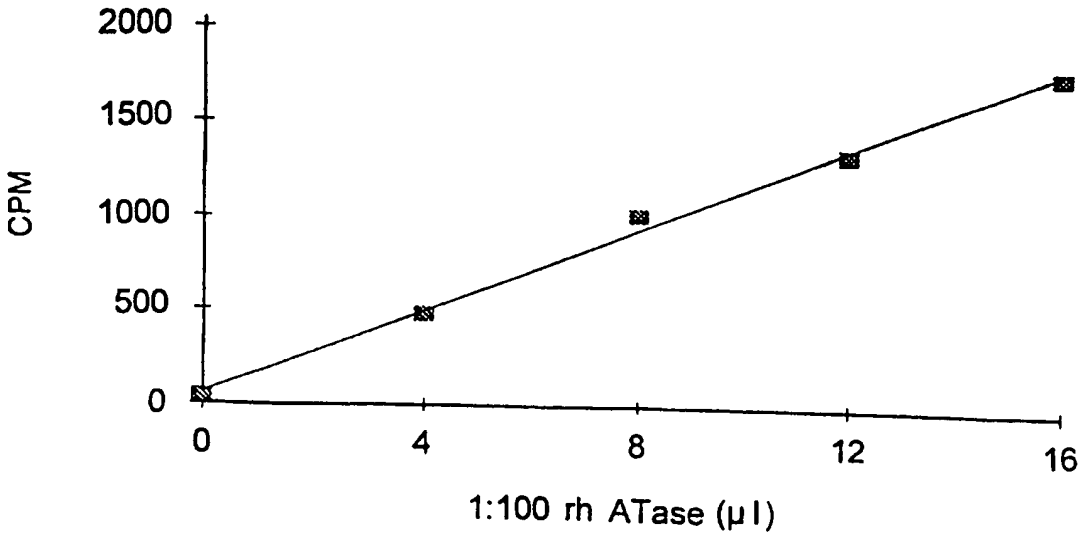

2000
1500
1000
500
0
CPM
0
4
8
12
16
1:100 rh ATase (µl)

FIG. 30

INTERNATIONAL SEARCH REPORT

International Application No
PCT/IE 96/00084

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07D473/18 C07D473/40 C07D251/52 A61K31/52 A61K31/505
C07D473/22 C07D239/30 C07H19/16 C07D498/04 C07D513/04
C07D471/04 C07D475/02 C07D409/12 C07D487/04

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07D C07H

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
6 A	CHEMICAL ABSTRACTS, vol. 101, no. 25, 17 December 1984 Columbus, Ohio, US; abstract no. 230466q, page 765; column R; XP002028572 see abstract & HETEROCYCLES, vol. 22, no. 8, 1984, pages 1789-1790, RAM SIYA ET AL: ---	2
6 X	THE JOURNAL OF ORGANIC CHEMISTRY, vol. 34, no. 7, July 1969, pages 2160-2163, XP002028568 MORRIS J. ROBINS ET AL: 'Page 2161; column 2: formula 13* --- -/--	2-43

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

1 Date of the actual completion of the international search
1 April 1997

Date of mailing of the international search report
09. 04. 97

Name and mailing address of the ISA
European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
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Fax (+ 31-70) 340-3016

Authorized officer
Luyten, H

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/IE96/00084

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 //(C07D487/04,249:00,239:00)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

	Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
1	X	JOURNAL OF MEDICINAL CHEMISTRY, vol. 38, no. 2, 20 January 1995, pages 359-365, XP002028569 MI-YOUNG CHAE ET AL: cited in the application ---	2-43
1	X	JOURNAL OF MEDICINAL CHEMISTRY, vol. 37, no. 3, 4 February 1994, pages 342-347, XP002028570 MI-YOUNG CHAE ET AL: cited in the application *Article* --- -/--	2-43

[X] Further documents are listed in the continuation of box C.

[ ] Patent family members are listed in annex.

* Special categories of cited documents:

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 N... 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

	Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
1	X	JOURNAL OF MEDICINAL CHEMISTRY, vol. 35, no. 23, 13 November 1992, pages 4486-4491, XP002028571 ROBERT C. MOSCHEL ET AL: cited in the application *Article*	1,2
1	X	WO 91 13898 A (THE UNITED STATES OF AMERICA) 19 September 1991 cited in the application see page 28 - page 32; claims	2-43
1	X	WO 94 29312 A (CANCER RESEARCH CAMPAIGN TECHNOLOGY LIMITED) 22 December 1994 cited in the application see the whole document	2-43
1	P,X	WO 96 04281 A (THE UNITED STATES OF AMERICA) 15 February 1996 cited in the application see the whole document	2-43

INTERNATIONAL SEARCH REPORT

International Application No.
PCT/IE 96/00084

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 35-36 are directed to a method of treatment of (diagnostic method practised on) the human/animal body the search has been carried out and based on the alleged effects of the compound/composition.

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Int[illegible]ication No
PCT/IE 96/00084

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9113898 A	19-09-91	US 5091430 A	25-02-92
		US 5352669 A	04-10-94
		AT 141925 T	15-09-96
		AU 646452 B	24-02-94
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		CZ 9503233 A	12-06-96
		EP 0702683 A	27-03-96
		FI 955906 A	02-02-96
		IE 62443 B	08-02-95
		JP 8511773 T	10-12-96
		NO 954985 A	07-02-96
		PL 311950 A	18-03-96
		SK 154795 A	03-07-96
		ZA 9404026 A	06-02-95
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		AU 3207995 A	04-03-96
		CA 2195856 A	15-02-96